# THE BACTERIA OF NEBRASKA SOIL

WITH SPECIAL REFERENCE

TO THE FIXATION OF NITROGEN, AMMONIFICATION
DENITRIFICATION IN NON-PROTEIN MEDIA,
INCLUDING OBSERVATIONS ON THE
REDUCTION OF NITRATES BY
SOIL BACTERIA IN
GENERAL

BY

JOHN J. PUTNAM

SUBMITTED TO THE

GRADUATE COLLEGE
OF
THE UNIVERSITY OF NEBRASKA

IN CANDIDACY FOR THE DEGREE
DOCTOR OF PHILOSOPHY

FEBRUARY 20, 1913

1913
THE WOODRUFF PRESS
LINCOLN, NEB.

Exchange

# THE BACTERIA OF NEBRASKA SOIL WITH SPECIAL REFERENCE TO THE FIXATION OF NITROGEN, AMMONIFICATION, DENITRIFICATION IN NON-PROTEIN MEDIA INCLUDING OBSERVATIONS ON THE REDUCTION OF NITRATES BY SOIL BACTERIA IN GENERAL

## HISTORICAL

This work was undertaken with the idea of ascertaining if possible, some of the many chemical changes taking place through the action of bacteria indigenous to Nebraska soil.

The fixation of nitrogen was first observed by M. Berthelot in 1885. He subsequently was able to prove that this phenomenon is not brought about exclusively by a purely chemical process, but is due to the activity of micro-organisms. The discovery of an anaerobic organism by S. Winogradsky in 1893, Clostridium pasteurianum, which he found fixed from 2.5 to 3 mg of nitrogen per gram of dextrose consumed, marked the first advance along this important line. Recent observers have added a few organisms to the list, Beyerinck, Löhnis and Lipman having labored successfully in this field.

In 1887 Schlossing and Muntz hazarded the opinion that the formation of nitrate within the soil is due to the vital activity of soil bacteria, and in a subsequent communication these two workers detailed some of the conditions requisite for the inception and course of nitrification. Much opposition developed from the advocates of the chemical theory. A reexamination of the comprehensive work of H. Plath by Londalt, who undertook the task in consequence of an objection raised by B. Frank, led to a complete confirmation of Plath's discoveries in all particulars. It was thus ascertained in 1888, by the exclusion method, that in the oxidation process now under our notice the role of oxygen-carrier is played by living organisms, and that nitrification consequently is a physiological process. The discovery and closer investigation of these unknown organisms was shortly afterwards effected by S. Wino-

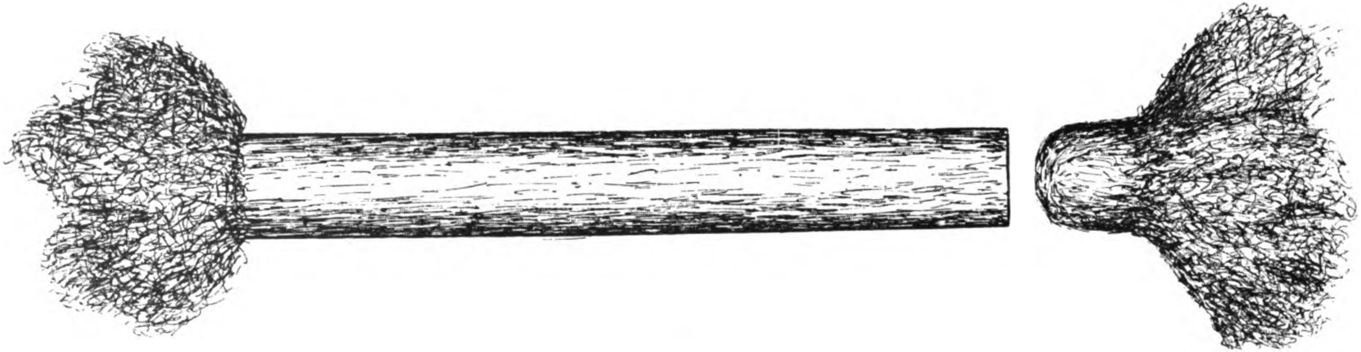
gradsky, who isolated them in pure culture. Of great importance is the fact determined by Winogradsky that the numerous species of the group of nitrifying bacteria may be classified into two sharply divided sub-groups: Nitroso-bacteria and Nitro-bacteria. The nitroso-bacteria oxidize ammonia to nitrous acid, while the nitro-bacteria lack the faculty of attacking ammonia, but perform the important task of converting nitrous acid into nitric acid.

We are indebted to E. Marchel for proving that the faculty of eliminating ammonia from albuminoids is common to many fungi. The potency of the different species was found by him to vary, the largest quantity being produced by Bacillus mycoides.

The first researches along the line of denitrification were undertaken by Jules Reiset in 1854 and 1855. He asserted that free nitrogen was always evolved during the decomposition of manure. Denitrification in arable soil was first noticed by Gappelsroder in 1862, and was long regarded as a purely chemical process. The first reference to the agency of bacteria in this decomposition was made by E. Mensel in 1875, and the earliest pure cultures of such organisms were obtained by U. Gayon and G. Dupetit in 1882. In succeeding years a large number of species, all capable of reducing nitrates, was made known. In 1888, P. Frankland was able to associate with the group in question 17 out of 32 species, and R. Warington 16 out of 25 species examined. A. Maassen, in 1902, found that out of 109 species, 85 were able to perform this function. But few of the organisms which have been observed to reduce nitrates to nitrites in pure culture, are able to continue the reduction to the liberation of free nitrogen.

---

For an inquiry into the various functions performed by soil bacteria in general and with reference to the factors concerned in the fixation of nitrogen by azotobacter, ammonification, reduction of nitrates and denitrification, in particular,seventy samples of soil were taken. These soils comprise perhaps all of the various types within our borders, with the possible exception of the alkali tracts which are interspersed over the western half of our state. Locations were made not with special reference to any favored locality or type of soil, but rather that the samples



Soil Tube.

should be fairly representative of the whole state. These samples were taken in tubes which were constructed especially for our purpose, and are of steel bicycle tubing, eight inches long and one inch in diameter. Whenever possible, the earth was removed in a crust of an inch or less in thickness, and the tube forced into the ground beneath, to a depth of four or five inches. About one inch of dirt was removed from the tube with a sterile knife, and the cotton plug readjusted. On being returned to the laboratory the samples were transferred to sterile four ounce salt-mouth bottles, thoroughly mixed, and soil taken therefrom as desired. Experience has abundantly proven that the whole process of sampling, transfer, etc., can be performed with such exactness that no contamination takes place.

The following list shows the character of each of the soils investigated:

| | | | | | |
|---|---|---|---|---|---|
| 1 | Fine sand loam | 25 | Silt loam | 49 | Muck |
| 2 | Fine sand loam | 26 | Silt loam | 50 | Loam |
| 3 | Fine sand loam | 27 | Loam | 51 | Loam |
| 4 | Fine sand loam | 28 | Loam | 52 | Silt loam |
| 5 | Fine sand loam | 29 | Loam | 53 | Loam |
| 6 | Fine sand loam | 30 | Loam | 54 | Clay loam |
| 7 | Fine sand loam | 31 | Fine sand loam | 55 | Silt loam |
| 8 | Fine sand loam | 32 | Loam | 56 | Silt loam |
| 9 | Fine sand loam | 33 | Loam | 57 | Calcareus clay |
| 10 | Fine sand loam | 34 | Loam | 58 | Fine sand loam |
| 11 | Fine sand loam | 35 | Loam | 59 | Silt loam |
| 12 | Fine sand loam | 36 | Loam | 60 | Fine sand loam |
| 13 | Fine sand loam | 37 | Loam | 61 | Fine sand loam |
| 14 | Fine sand loam | 38 | Loam | 62 | Gravel |
| 15 | Fine sand loam | 39 | Loam | 63 | Fine sand loam |
| 16 | Silt loam | 40 | Silt loam | 64 | Fine sand loam |
| 17 | Silt loam | 41 | Loam | 65 | Fine sand loam |
| 18 | Silt loam | 42 | Loam | 66 | Fine sand loam |
| 19 | Fine sand loam | 43 | Loam | 67 | Fine sand loam |
| 20 | Fine sand loam | 44 | Silt loam | 68 | Calcareus loam |
| 21 | Silt loam | 45 | Fine sand loam | 69 | Fine sand loam |
| 22 | Silt loam | 46 | Fine sand loam | 70 | Fine sand loam |
| 23 | Silt loam | 47 | Silt loam | | |
| 24 | Silt loam | 48 | Muck | | |

No more samples were taken at one time than could be handled with promptness, therefore necessitating several trips over the state.

In order to approximate the number of bacteria within the soil, the seventy samples were plated both on nutrient agar, and Ashby's medium to which was added fifteen grams of agar-agar per liter.

Nutrient medium recommended by Ashby for fixation of nitrogen by azotobacter:

| | |
|---|---|
| Mannite | 20.00 grams |
| Di-potassium phosphate | 0.20 gram |
| Magnesium sulphate | 0.20 gram |
| Sodium chloride | 0.20 gram |
| Calcium sulphate | 0.10 gram |
| Calcium carbonate | 5.00 grams |
| Distilled water | 1000.00 grams |

A Kjeldahl determination disclosed the fact that good agar-agar contained 0.16% of nitrogen, or a little below the average of good soil. Therefore, each plate of the Ashby medium contained approximately .25 mg of nitrogen, or about .0025%, which is in the neighborhood of one one-hundredth the nitrogen content of a good loam.

A little nitrogen must inevitably be carried over in the process of dilution, etc.

The plates were counted after five days incubation at room temperature, which prevailed at about 33°C.

The figures in the adjoining table represent the count per gram of soil dried at 100-110°C, to constant weight.

## NUMBER OF BACTERIA PER GRAM OF SOIL DRIED TO CONSTANT WEIGHT AT 100-110°C

TABLE I

| Soil No. | Nutrient agar | Ashby's medium | Moisture Per cent |
|---|---|---|---|
| 1 | 693,000 | 1,155,000 | 13.44 |
| 2 | 94,900 | 940,000 | 5.23 |
| 3 | 3,301,000 | 3,056,000 | 18.22 |
| 4 | 1,673,000 | 3,705,000 | 16.33 |
| 5 | 1,793,000 | 2,577,000 | 10.77 |
| 6 | 210,000 | 1,053,000 | 5.09 |
| 7 | 288,000 | 2,138,000 | 6.41 |
| 8 | 381,000 | 448,000 | 10.83 |
| 9 | 1,353,000 | 1,466,000 | 11.37 |

TABLE I—Continued

| Soil No. | Nutrient agar | Ashby's medium | Moisture Per cent |
|---|---|---|---|
| 10 | 2,407,000 | 150,000 | 16.91 |
| 11 | 224,000 | 693,000 | 6.27 |
| 12 | 153,000 | 54,800 | 8.91 |
| 13 | 15,300 | 404,000 | 13.50 |
| 14 | 75,800 | 162,000 | 7.73 |
| 15 | 289,000 | 133,000 | 13.55 |
| 16 | 34,000 | 10,600 | 5.95 |
| 17 | 42,900 | 219,000 | 44.45 |
| 18 | 790,000 | 11,900 | 16.56 |
| 19 | 50,600 | 562,000 | 11.09 |
| 20 | 115,000 | 399,000 | 4.98 |
| 21 | 1,530,000 | 3,178,000 | 15.05 |
| 22 | 737,000 | 1,989,000 | 9.15 |
| 23 | 474,000 | 830,000 | 15.68 |
| 24 | 946,000 | 2,601,000 | 15.44 |
| 25 | 165,000 | 1,650,000 | 9.54 |
| 26 | 82,000 | 671,000 | 15.11 |
| 27 | 65,000 | 412,000 | 7.96 |
| 28 | 293,000 | 1,084,000 | 11.44 |
| 29 | 215,000 | 420,000 | 7.15 |
| 30 | 135,000 | 135,200 | 9.80 |
| 31 | 59,000 | 462,000 | 15.59 |
| 32 | 40,700 | 184,000 | 14.23 |
| 33 | 639,000 | 575,000 | 6.15 |
| 34 | 713,000 | 594,000 | 7.56 |
| 35 | 409,000 | 301,000 | 7.19 |
| 36 | 445,000 | 434,000 | 7.95 |
| 37 | 574,000 | 1,768,000 | 9.55 |
| 38 | 1,373,000 | 2,146,000 | 6.84 |
| 39 | 784,000 | 753,000 | 5.44 |
| 40 | 389,000 | 811,000 | 5.11 |
| 41 | 716,000 | 791,000 | 6.49 |
| 42 | 236,000 | 381,000 | 6.97 |
| 43 | 1,795,000 | 2,218,000 | 5.82 |
| 44 | 731,000 | 1,801,000 | 11.19 |
| 45 | 637,000 | 1,880,000 | 4.28 |
| 46 | 416,000 | 420,000 | 3.89 |
| 47 | 873,000 | 484,000 | 7.15 |
| 48 | 1,886,000 | 551,000 | 31.08 |
| 49 | 1,965,000 | 573,000 | 38.95 |
| 50 | 335,000 | 1,300,000 | 7.71 |
| 51 | 364,000 | 1,287,000 | 6.79 |
| 52 | 74,000 | 391,000 | 5.43 |

**TABLE I—Concluded**

| Soil No. | Nutrient Agar | Ashby's Medium | Moisture Per cent |
|---|---|---|---|
| 53 | 254,000 | 637,000 | 5.91 |
| 54 | 255,000 | 210,000 | 9.79 |
| 55 | 1,602,000 | 554,000 | 16.98 |
| 56 | 1,006,000 | 524,000 | 8.56 |
| 57 | 11,000 | 4,500 | 9.70 |
| 58 | 818,000 | 549,000 | 7.16 |
| 59 | 506,000 | 60,000 | 5.21 |
| 60 | 56,500 | 9,200 | 9.88 |
| 61 | 4,224,000 | 833,000 | 12.41 |
| 62 | 10 | 000 | 4.48 |
| 63 | 468,000 | 60,300 | 3.90 |
| 64 | 947,000 | 904,000 | 7.12 |
| 65 | 158,000 | 70,000 | 4.30 |
| 66 | 134,000 | 17,000 | 11.98 |
| 67 | 40,000 | 30,700 | .84 |
| 68 | 10,008,000 | 512,000 | 16.07 |
| 69 | 380,000 | 83,600 | 7.93 |
| 70 | 1,850,000 | 103,000 | 8.18 |

In forty of the samples the number of bacteria which developed visible colonies on the non-proteid medium were in excess, and in many instances in great excess, of those on the nutrient agar.

It will be observed that in the remaining thirty samples the number of colonies in the Ashby medium very closely approximate those on the nutrient agar, and in but few instances were the colonies on nutrient agar in great preponderance.

As to what is to be inferred from this it is difficult to conjecture; nor can we conclude that we have here two distinct flora.

One striking feature which invites attention to the Ashby plate, is the variety of pigments observable. Especially characteristic are the blue, violet, pink, purple, red and brown colors which develop after several days.

Cladothrix dichotoma, which will be considered later, is one of the most common soil organisms which thrive on a limited nitrogen supply. It is a matter of common knowledge that organisms growing under adverse conditions, or rather in

an environment other than the optimum, lose at least some of their distinguishing characteristics. Many of these organisms, when transferred to nutrient agar slants, grow vigorously without pigment production.

The azotobacter develop on the Ashby medium in great profusion, however, the differentiation of the nitrogen-fixing bacteria is not sufficiently established to render an enumeration of them possible.

## THE FIXATION OF FREE NITROGEN BY BACTERIA

The relation of bacteria to nitrogen is perhaps the most important problem which presents itself to the agriculturist; the reason being that while the nitrogen forms a very large proportion of the constituents necessary to the building up of plant tissue, it is present in the soil in a very limited quantity, and consequently constant cropping would tend toward exhausting the supply.

The fixation of free nitrogen by bacteria is consummated in two widely different ways, commonly designated as the symbiotic and non-symbiotic relation. Symbiosis involves a favorable influence of one species upon another. Many observers contend that this symbiotic relation is detrimental to the host. The symbiotic relation existing between the leguminoceae and certain bacteria enables the former to absorb free nitrogen from the air and elaborate it into nitrogenous compounds. This metamorphosis takes place within the leguminous nodules, the earliest description of which was given by Malpighi in 1687, and this observer referred to them as galls, *i. e.*, diseased excresences, an opinion also shared by later writers.

Treviranus, in 1853, was the first to regard these nodules as normal growths, and thirteen years later they were studied by Woronin, who made the subsequently important observation, that the formation contains entirely closed cells filled with bacteria.

Beyerinck, in 1888, indubitably established the fungoid nature of these bacteria by isolating them from the nodules, and cultivating them further in artificial media. Some exhibited certain slight but undeniable differences which were not so extensive as to make their discoverer feel justified in

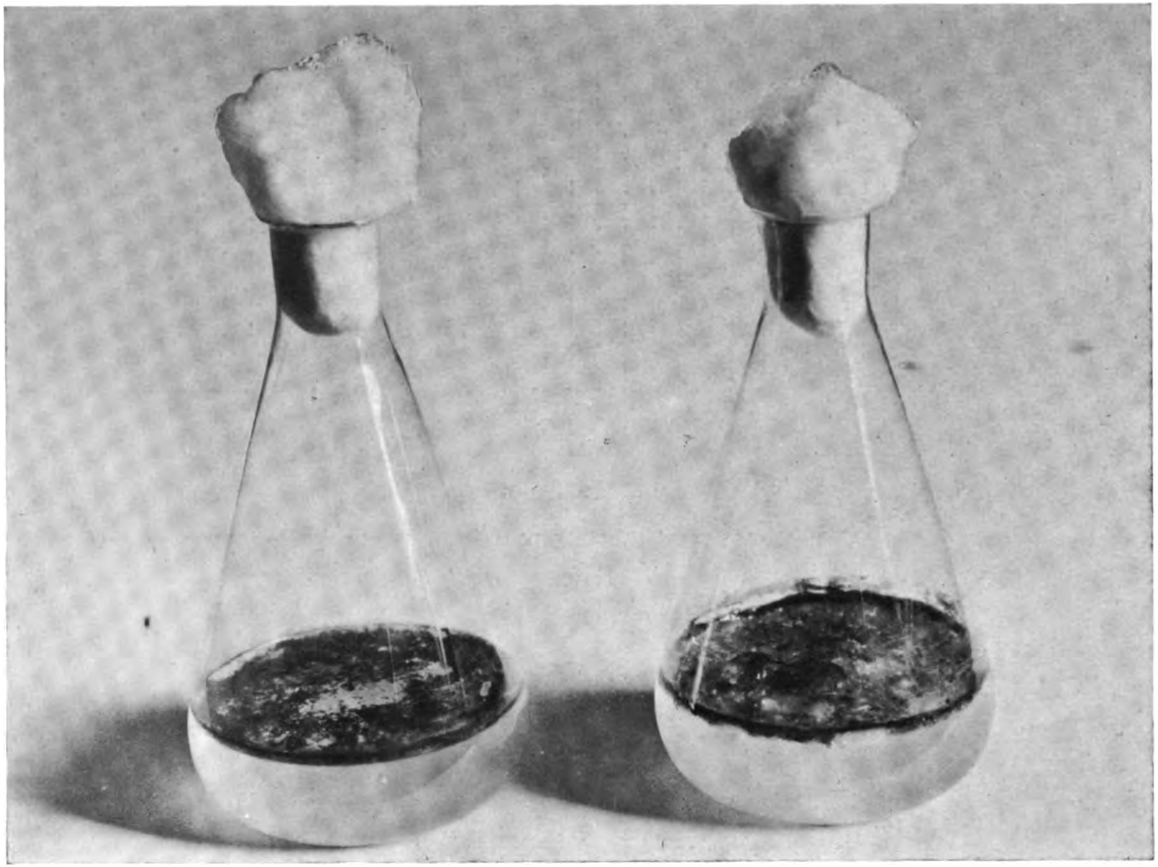
classifying the organisms as separate species. Beyerinck proposed the name Bacillus radicicola, for these nodule producing bacteria; as to whether there are more than one species, authorities are still undetermined. Hereby is evolved a rational system for the continuous addition of nitrogen to the soil, an increase which can not only be enjoyed and appropriated by the leguminous plants, but likewise by succeeding vegetable growth.

The non-symbiotic fixation of nitrogen possesses the important feature of having more universal application. The following aerobic species are the most vigorous nitrogen fixing organisms hitherto discovered: A. agilis, A. chroococcum, A. vinelandi, A. beyerincki, A. vitreum, and A. woodstowni. Of these A. chroococcum is in all probability the most common in our soil. I have isolated this organism from many parts of the state.

## STUDIES ON IMPURE CULTURES

In order to determine the relative nitrogen-fixing power of our soils, the aforementioned samples were inoculated into Ashby's medium, and the following process and technique followed: One hundred cubic centimeters of Ashby's medium was measured into 250 cc Erlenmeyer flasks, and sterilized: these flasks, therefore, each contained two grams of mannite; the medium occupying about three-fourths of an inch in depth in the bottom of the flask, there remained above the surface an abundant air space. These flasks were each inoculated with one gram of soil, and incubated at room temperature, which prevailed at about 33°C, for twenty-one days. At the end of this period the entire contents of these flasks were transferred to the Kjeldahl apparatus and the total nitrogen content determined by the Kjeldahl method, the ammonia being distilled over into tenth-normal sulphuric acid and titrated back with tenth-normal sodium hydroxide, using congo red as indicator. The original nitrogen content of the soil was determined by the Kjeldahl method, using ten grams of sample.

Each operation during the investigation was carefully checked in order to reduce the possible error to the limit of experimental manipulation. Samples number 48 and 49 were



Azotobacter chroococcum from Nebraska soil.

the only typical muck soils available. It will be observed that these show a fixation of 4.38 and 5.02 mg respectively. One mg of nitrogen at 0°C and 760 mm pressure represents approximately 0.80 cubic centimeters. The per cent of nitrogen determined for these soils dried to constant weight, at 100-110°C, were .4331 and .5481, which is very greatly in excess of any of the remaining soils. The average of sixteen soils of which the nitrogen content ranged uniformly above .2024 per cent, with a limit of .5481 was 4.91 mg, while the average of sixteen soils which fixed more than 4.91 mg and which had a nitrogen content of uniformly less than .2024 per cent, was 6.72 mg. Soil number 1 which had a nitrogen content of .0926 per cent, fixed 10.74 mg which was the highest value of any. It is reasonable, therefore, to conclude that a soil which contains much above .1000 per cent of nitrogen, other things being favorable, may equal or surpass any other soil in nitrogen fixing possibilities. Probably the number of azotobacter present in the soil determined the speed of the reaction.

Azotobacter chroococcum was found to be universally distributed over the state. Many of the cultures which evinced strong nitrogen-fixing properties were covered with an imperfect floating membrane of brownish color shading off to almost black.

A. chroococcum was definitely isolated from an alfalfa field at a depth of three feet, to which particular reference will be made later.

Fungus growth developed on the surface of the medium in some instances. It is significant that in those overrun with molds and similar vegetation, the liquid frequently exhibited decided colors, usually yellow, though in one instance pink.

The following table shows the amount of nitrogen fixed in milligrams and the percentage of moisture and nitrogen in each of the seventy samples of soil investigated:

# FIXATION OF NITROGEN

## ASHBY'S MEDIUM

### MANNITE

### TABLE II

| Soil No. | Moisture Per cent | N Fixed in Mg | Per cent N in Sample |
|---|---|---|---|
| 1 | 13.44 | 10.74 | .0926 |
| 2 | 5.23 | 3.70 | .1981 |
| 3 | 18.22 | 6.05 | .2507 |
| 4 | 16.33 | 4.98 | .1724 |
| 5 | 10.77 | 3.37 | .1104 |
| 6 | 5.09 | 3.04 | .1093 |
| 7 | 6.41 | 9.76 | .1609 |
| 8 | 10.83 | 7.22 | .1987 |
| 9 | 11.37 | 6.94 | .1807 |
| 10 | 16.91 | 5.60 | .2067 |
| 11 | 6.27 | 4.50 | .1537 |
| 12 | 8.91 | 4.97 | .1528 |
| 13 | 13.50 | 0.12 | .1487 |
| 14 | 7.73 | 4.17 | .1493 |
| 15 | 13.55 | 4.08 | .2335 |
| 16 | 5.95 | 0.19 | .1503 |
| 17 | 4.45 | 2.58 | .1418 |
| 18 | 16.56 | 5.57 | .1549 |
| 19 | 11.09 | 5.19 | .1441 |
| 20 | 4.98 | 2.70 | .1431 |
| 21 | 15.05 | 6.32 | .2176 |
| 22 | 9.15 | 5.50 | .2092 |
| 23 | 15.69 | 4.56 | .2507 |
| 24 | 15.44 | 7.30 | .2351 |
| 25 | 9.54 | 4.56 | .2254 |
| 26 | 15.11 | 3.66 | .2024 |
| 27 | 7.96 | 0.59 | .2110 |
| 28 | 11.44 | 4.59 | .2063 |
| 29 | 7.15 | 4.63 | .2156 |
| 30 | 9.80 | 0.61 | .1731 |
| 31 | 15.59 | 0.63 | .1725 |
| 32 | 14.23 | 0.29 | .1686 |
| 33 | 6.15 | 2.83 | .1241 |
| 34 | 7.56 | 6.18 | .2590 |
| 35 | 7.18 | 4.16 | .1462 |
| 36 | 7.95 | 3.95 | .1730 |
| 37 | 9.55 | 5.14 | .1949 |

TABLE II—Continued

| Soil No. | Moisture Per cent | N Fixed in Mg | Per cent N in Sample |
|---|---|---|---|
| 38 | 6.84 | 4.26 | .1683 |
| 39 | 5.44 | 2.74 | .1720 |
| 40 | 5.11 | 5.77 | .1667 |
| 41 | 6.49 | 5.39 | .1852 |
| 42 | 6.97 | 2.21 | .1477 |
| 43 | 5.32 | 8.57 | .1745 |
| 44 | 11.19 | 4.23 | .1800 |
| 45 | 4.28 | 7.72 | .1153 |
| 46 | 3.89 | 7.66 | .1028 |
| 47 | 7.15 | 3.41 | .1274 |
| 48 | 31.08 | 4.38 | .4331 |
| 49 | 38.95 | 5.02 | .5481 |
| 50 | 7.71 | 4.33 | .1574 |
| 51 | 6.79 | 4.93 | .1876 |
| 52 | 5.43 | 0.15 | .1381 |
| 53 | 5.81 | 2.63 | .1371 |
| 54 | 9.79 | 0.29 | .0472 |
| 55 | 16.98 | 5.65 | .2031 |
| 56 | 8.56 | 4.07 | .1016 |
| 57 | 9.70 | 0.00 | .0172 |
| 58 | 7.16 | 4.06 | .0722 |
| 59 | 5.21 | 0.13 | .0875 |
| 60 | 9.88 | 0.00 | .0176 |
| 61 | 12.41 | 6.84 | .1831 |
| 62 | 4.48 | 0.00 | .0051 |
| 63 | 3.90 | 1.36 | .0425 |
| 64 | 7.12 | 0.00 | .0911 |
| 65 | 4.30 | 0.57 | .1006 |
| 66 | 11.98 | 0.00 | .0347 |
| 67 | .84 | 0.24 | .0308 |
| 68 | 16.07 | 3.87 | .1604 |
| 69 | 7.93 | 2.78 | .0598 |
| 70 | 8.13 | 5.17 | .0892 |

## THE AVAILABILITY OF VARIOUS COMPOUNDS EFFECTING NITROGEN FIXATION

It having been previously established that carbohydrates were essential for the maximum efficiency of nitrogen fixation, many sugars have been studied in these investigations. In this connection I have employed the following: mannite, maltose, lactose, saccharose, dextrose, galactose, levulose, arabinose, dulcite, sorbit, raffinose, rhamnose, mannose, erythrite, xylose, quercit, glycerine, dextrin, inulin, calcium lactate, and calcium butyrate. These compounds were the best obtainable, mostly Kahlbaum's product, and were accurately assayed for nitrogen.

Ten soils which showed good fixation on mannite were selected for this purpose: numbers 1, 2, 7, 10, 24, 34, 41, 43, 47 and 61. These soils were inoculated into Ashby's medium under conditions similar to those followed in the previous experiment, with the one exception that the mannite was replaced by a special sugar or other compound. While it would have been highly desirable to have the data for all the sugars on the ten samples, the prohibitive price on many rendered this quite impossible. An inspection of the table shows the ten highest averages as follows:

| | | | |
|---|---|---|---|
| Sorbit | 8.32 mg | Dulcite | 6.21 mg |
| Mannite | 7.17 mg | Arabinose | 6.14 mg |
| Maltose | 6.34 mg | Dextrose | 5.32 mg |
| Mannose | 6.32 mg | Galactose | 5.08 mg |
| Levulose | 6.28 mg | Rhamnose | 4.92 mg |

The position held by sorbit is probably only possible because of the remarkable soil number 7. Of the disaccharides, maltose gave the best results, lactose second, and saccharose third.

An impure sample of maltose which we had in the laboratory, and which contained 15 mg of nitrogen per two grams of sugar, fixed an average on soils 2, 10, and 41 of 1.02 mg, while the same soils on pure maltose corrected for a very small percentage of nitrogen, fixed an average of 4.97 mg. This may be accepted as additional testimony that the presence of nitrogenous compounds in considerable amounts is not conducive to high fixation. Mannose, the aldehyde of the alcohol man-

nite, might be expected to approach the latter in fixation, but this did not prove to be the case, yet it differed from maltose only in the second place of decimals. Erythrite fixed an average, on soils 1 and 43 in twenty-one days, of 0.18 mg, while on soils 10, 24, 34 and 41, in fifty-four days, an average of 5.59 mg was fixed. The slow fermentation of this sugar renders it useless for laboratory purposes. Dextrin and inulin gave comparable results which were inconsiderable. Probably twenty-one days is insufficient to develop the maximum efficiency of these polysaccharides.

Glycerine in soils 1 and 43 fixed an average of 1.13 mg in twenty-one days. Soils 7, 10, 24, 34, 41, 47, and 61, fixed an average of 6.64 mg in thirty-nine days. Soil number 1 fixed 3.58 mg in thirty-nine days and soil 43 fixed 4.74 mg in the same time, a gain in the first instance of 2.61 mg and in the second of 2.45 mg in eighteen days. The slow fermentation of glycerine relegates it to the class with erythrite.

In the work on mannite solutions one is struck with the great variety of odors, but perhaps the most characteristic is that of butyric acid. This led me to conclude that butyric acid or oxybutric was either one of the splitting products of mannite, or that according to an early discovery, two molecules of lactic acid were changed to one of butyric acid, giving off two molecules of carbon dioxide and two molecules of hydrogen. After adding calcium butyrate to Ashby's medium it was inoculated with soils 1, 10 and 43, these yielded an average fixation of 0.15 mg. The butyrate therefore seemed not available for carbon supply. In place of calcium butyrate, calcium lactate was next introduced using three grams to the flask, an equivalent of 2.45 grams calculated as free lactic acid. The average fixation for ten soils was 3.01 mg. The figures on this compound do not show the uniformity of the others, although soils 2, 7, 47 and 61 did remarkably well. The odor of butyric acid was not so pronounced as had been expected, but some cultures showed unmistakable evidence of its presence.

In the fermentation of mannite considerable ethyl alcohol is split off. An analysis of the total acidity revealed approximately 30% acetic acid and 70% butyric acid.

The following table shows the amount of nitrogen fixed when grown in a medium containing the compounds listed:

## THE AVAILABILITY OF VARIOUS COMPOUNDS FOR NITROGEN FIXATION

TABLE III

| Soils | 1 | 2 | 7 | 10 | 24 | 34 | 41 | 43 | 47 | 61 |
|---|---|---|---|---|---|---|---|---|---|---|
| Maltose.... | 5.24 | 4.06 | 11.21 | 4.92 | 4.53 | 5.70 | 5.93 | 4.40 | 5.79 | 11.61 |
| Lactose.... | 3.68 | 2.48 | ..... | 5.36 | 4.47 | 4.94 | 4.21 | 2.24 | 7.60 | 7.62 |
| Saccharose.. | 3.98 | 3.57 | 3.44 | 5.48 | 3.75 | 3.26 | 3.45 | 2.98 | 3.65 | 5.79 |
| Mannite.... | 10.74 | 3.70 | 9.76 | 5.60 | 7.30 | 6.18 | 5.39 | 8.57 | 7.66 | 6.84 |
| Mannose... | 11.10 | 3.50 | 10.90 | 4.57 | 3.35 | 5.94 | 4.91 | 5.58 | 10.31 | 3.01 |
| Dextrose... | 4.85 | 3.62 | ..... | 5.86 | 4.75 | 3.36 | 4.77 | 4.41 | 4.32 | 12.02 |
| Levulose.... | 4.84 | 4.55 | 9.82 | 6.62 | 5.00 | 4.80 | 6.94 | 5.98 | 6.64 | 7.69 |
| Galactose... | 5.87 | 3.17 | ..... | 4.78 | 4.42 | 5.35 | 4.36 | 3.74 | 7.48 | 6.56 |
| Raffinose... | 5.57 | 3.39 | 5.71 | 4.47 | 4.03 | 6.44 | 5.54 | 4.45 | ..... | 4.53 |
| Rhamnose.. | 5.06 | 4.66 | ..... | 3.94 | 5.60 | ..... | 5.61 | 4.43 | ..... | 5.15 |
| Arabinose... | 6.41 | ..... | 6.80 | 5.52 | 5.02 | 5.76 | 7.30 | 6.23 | ..... | ..... |
| Dulcite..... | 3.28 | ..... | 14.35 | 5.55 | 4.69 | 5.34 | 5.32 | 5.00 | ..... | ..... |
| Erythrite... | 0.23 | ..... | ..... | 7.83 | 4.02 | 8.07 | 2.45 | 0.13 | ..... | ..... |
| Dextrin.... | 3.70 | 1.88 | ..... | 3.52 | ..... | ..... | 3.64 | 2.02 | ..... | ..... |
| Inulin...... | 3.68 | 2.28 | ..... | 2.02 | ..... | ..... | 2.86 | 4.05 | ..... | ..... |
| Sorbite..... | 8.13 | ..... | 13.27 | ..... | ..... | ..... | ..... | 3.57 | ..... | ..... |
| Xylose..... | ..... | ..... | ..... | ..... | ..... | 6.93 | 3.84 | 3.42 | ..... | ..... |
| Quercit..... | ..... | ..... | ..... | ..... | 3.29 | ..... | ..... | ..... | ..... | ..... |
| Glycerine... | 3.58 | ..... | 3.37 | 6.50 | 6.90 | 6.53 | 7.90 | 4.74 | 6.14 | 9.18 |
| Calcium Lactate..... | 1.24 | 3.39 | 3.90 | 2.13 | 0.88 | 2.97 | 0.93 | 2.55 | 4.53 | 7.59 |

## STUDIES ON AMMONIFICATION IN MIXED CULTURE

The original 70 samples were used in connection with this experiment. The medium consisted of a solution of ten grams of Witte's peptone per liter of distilled water. One hundred cubic centimeters of this solution were measured into flasks of 500 cc capacity, sterilized, and inoculated with one gram of soil. After seven days incubation at 33°C, the contents of these Erlenmeyer flasks were transferred to the Kjeldahl apparatus, ten grams of magnesium oxide added, and the ammonia distilled over into semi-normal hydrochloric acid, and titrated back with semi-normal ammonium hydroxide, using congo red as indicator. In order to ascertain the percentage of nitrogen in the peptone, a composite sample was taken from the thirteen stock bottles, intimately mixed, and run by

the Kjeldahl method in triplicate. This composite sample which assayed 15.567% nitrogen was used in all ammonification experiments. Each flask therefore contained 155.67 mg of nitrogen. It will be observed that in soils number 49 and 61, over 80% of the nitrogen was evolved as ammonia. The muck soil 49 being a little below the loam. A survey of the table indicates that those soils which were especially active in fixing nitrogen also converted into ammonia more than 70% of the available nitrogen.

## AMMONIFICATION * IMPURE CULTURES

TABLE IV

| Soil No. | Nitrogen Evolved as Ammonia in Mg | Per cent of Nitrogen Evolved as Ammonia |
|---|---|---|
| 1 | 117.26 | 75.32 |
| 2 | 105.77 | 67.94 |
| 3 | 122.80 | 78.88 |
| 4 | 116.00 | 74.51 |
| 5 | 120.13 | 77.16 |
| 6 | 115.03 | 73.88 |
| 7 | 110.47 | 70.96 |
| 8 | 120.34 | 77.30 |
| 9 | 121.75 | 78.21 |
| 10 | 118.87 | 76.26 |
| 11 | 109.77 | 70.51 |
| 12 | 96.11 | 61.73 |
| 13 | 106.67 | 68.53 |
| 14 | 96.67 | 62.09 |
| 15 | 111.66 | 71.72 |
| 16 | 106.19 | 68.21 |
| 17 | 112.30 | 72.13 |
| 18 | 113.13 | 72.67 |
| 19 | 106.61 | 68.48 |
| 20 | 86.02 | 55.25 |
| 21 | 108.65 | 69.79 |
| 22 | 108.51 | 69.70 |
| 23 | 109.00 | 70.01 |
| 24 | 109.00 | 70.01 |
| 25 | 102.97 | 66.14 |
| 26 | 103.25 | 66.32 |
| 27 | 102.62 | 65.92 |
| 28 | 102.97 | 66.14 |

TABLE IV—Continued

| Soil No. | Nitrogen Evolved as Ammonia in Mg | Per cent of Nitrogen Evolved as Ammonia |
|---|---|---|
| 29 | 93.45 | 60.03 |
| 30 | 93.80 | 60.25 |
| 31 | 115.58 | 74.24 |
| 32 | 110.12 | 70.74 |
| 33 | 118.66 | 76.22 |
| 34 | 120.55 | 77.50 |
| 35 | 121.19 | 77.85 |
| 36 | 117.68 | 75.59 |
| 37 | 117.26 | 75.32 |
| 38 | 112.22 | 72.08 |
| 39 | 113.48 | 73.89 |
| 40 | 102.76 | 66.01 |
| 41 | 122.10 | 78.43 |
| 42 | 118.94 | 76.40 |
| 43 | 110.75 | 71.14 |
| 44 | 120.06 | 77.12 |
| 45 | 110.33 | 70.87 |
| 46 | 107.81 | 69.25 |
| 47 | 110.54 | 71.00 |
| 48 | 113.84 | 73.12 |
| 49 | 126.09 | 80.99 |
| 50 | 99.68 | 64.03 |
| 51 | 118.59 | 76.18 |
| 52 | 111.59 | 71.68 |
| 53 | 100.95 | 64.84 |
| 54 | 84.76 | 54.44 |
| 55 | 98.84 | 63.49 |
| 56 | 77.75 | 49.93 |
| 57 | 82.03 | 52.69 |
| 58 | 92.89 | 59.66 |
| 59 | 101.29 | 65.06 |
| 60 | 34.60 | 22.22 |
| 61 | 126.44 | 81.22 |
| 62 | 37.40 | 24.02 |
| 63 | 71.58 | 45.98 |
| 64 | 92.03 | 59.75 |
| 65 | 79.58 | 51.11 |
| 66 | 27.04 | 17.36 |
| 67 | 99.54 | 63.94 |
| 68 | 105.63 | 67.85 |
| 69 | 94.64 | 60.79 |
| 70 | 95.90 | 61.60 |

## THE AMMONIFICATION OF MEAT-EXTRACT

As a comparison between the availability of meat-extract and peptone nitrogen for ammonification experiments, a medium consisting of 19.85 grams Liebig's extract of meat per liter of distilled water, was used. The meat-extract assayed 7.94% nitrogen, which was approximately the peptone-nitrogen content.

## TABLE SHOWING COMPARATIVE WEIGHTS OF NITROGEN EVOLVED AS AMMONIA IN PEPTONE AND MEAT-EXTRACT MEDIA HAVING SAME NITROGEN CONTENT

Table V

| Soil No. | Peptone | | Meat-Extract | | Per cent Difference |
|---|---|---|---|---|---|
| | Mg N Evolved | Per cent N Evolved | Mg N Evolved | Per cent N Evolved | |
| 9....... | 104.23 | 66.10 | 121.75 | 78.21 | 12.11 |
| 37....... | 107.03 | 67.87 | 117.26 | 75.32 | 7.45 |
| 43....... | 92.74 | 58.81 | 110.75 | 71.14 | 12.33 |
| 49....... | 107.73 | 68.32 | 126.09 | 80.99 | 12.67 |
| 61....... | 120.63 | 76.50 | 126.44 | 81.22 | 4.72 |

The ammonification in the meat-extract medium appears to be uniformly lower than in the peptone solution, and the difference in per cent in soils 9, 43 and 49 is constant. An attempt was made to use lecithin as a culture medium for similar experiments, but the nitrogen content being low (1.80)%, and the insolubility so great, the attempt was finally abandoned.

## THE DEVIATION OF NASCENT HYDROGEN FROM THE PROTEIN NITROGEN EFFECTED BY THE PRESENCE OF NITRATES AND NITRITES

Fifteen flasks each containing 100 cc of the 1% peptone solution, were inoculated with one gram of soil as indicated: To each of the second set of five, was added one gram of potassium nitrate. Similarly to the third set of five, was added

one gram of potassium nitrite. These flasks were incubated at 33°C for seven days, the contents then transferred to the Kjeldahl apparatus and the ammonia determined as in the previous work.

TABLE VI

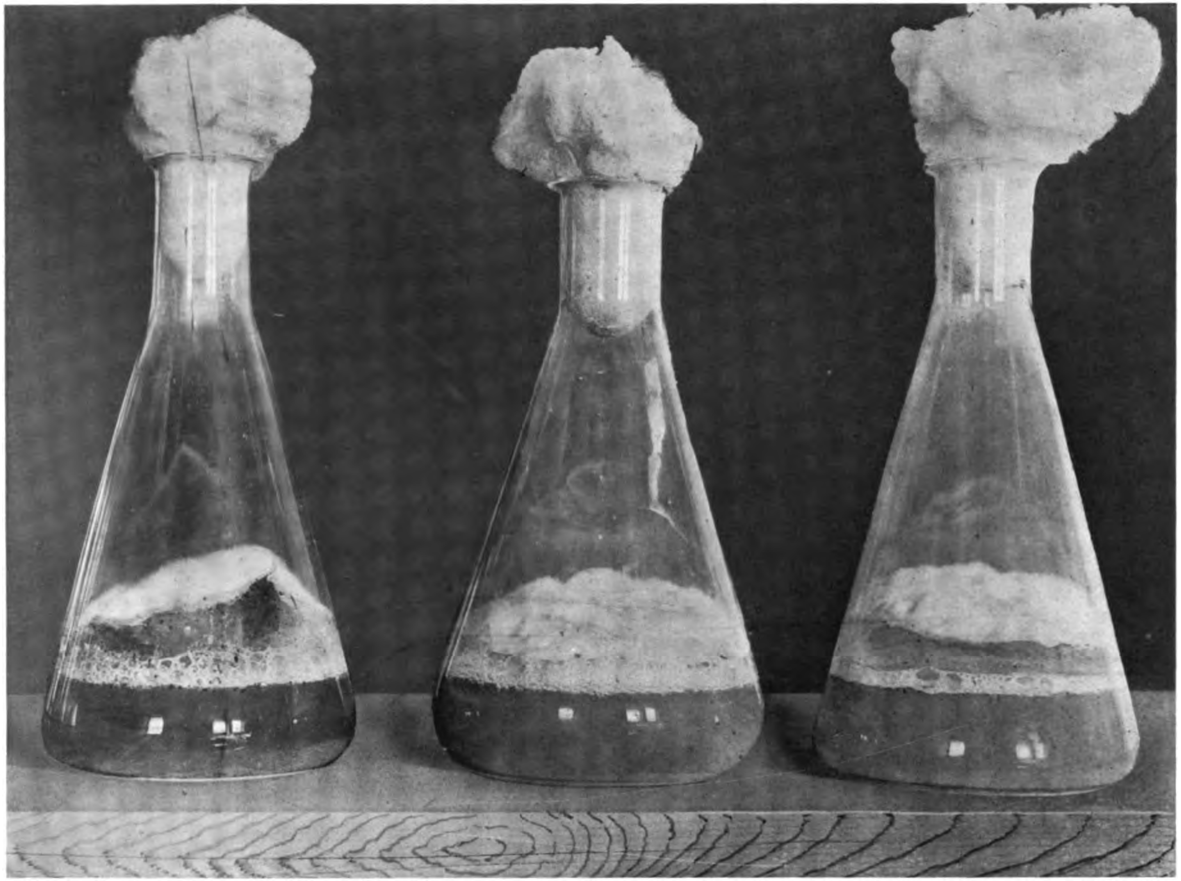
| | Soil No. | | | | |
|---|---|---|---|---|---|
| | 9 | 37 | 43 | 49 | 61 |
| Peptone | 72.40 | 65.92 | 72.76 | 76.49 | 74.74 |
| Peptone plus pot. nitrate | 39.86 | 32.80 | 40.90 | 36.26 | 43.87 |
| Peptone plus pot. nitrite | 31.18 | 25.96 | 33.97 | 22.67 | 30.91 |

The figures in the above table represent the percentage of nitrogen evolved as ammonia. The conclusion is inevitable that considerable quantities of nitrates or nitrites cannot exist in the soil together with a high percentage of protein nitrogen. The ammonification equilibrium is likewise disturbed by an excess of nitrates or nitrites.

The figures in the above table seem to indicate that the hydrogen together with a large proportion of the nitrogen, perhaps partly because of the violence of the reaction, on being set free escapes as free hydrogen and nitrogen. We have in each column of the table a consistently decreasing percentage of ammonification. The relation between the peptone and peptone-nitrate being uniformly greater than the relation between the peptone-nitrate and peptone-nitrite. This uniformity is pronounced.

## STUDIES ON THE REDUCTION OF NITRATES

IMPURE CULTURES: One hundred cubic centimeters of Ashby's medium without the carbohydrate, was measured into Erlenmeyer flasks of 250 cc capacity. To each flask was added varying amounts of mannite, dextrose and potassium salts as indicated in the table. These flasks were inoculated with different soils and kept at 28°C.



Denitrification in Ashby's medium: showing vigorous reaction.

TABLE VII

| Soil No. | Mannite | $KNO_3$ | Day on which Nitrate was Found to be Reduced |
|---|---|---|---|
| 5 | 2 grams | 1 gram | 5th |
| 10 | 2 grams | 1 gram | 5th |
| 19 | 2 grams | 1 gram | 5th |
| 22 | 2 grams | 1 gram | 5th |
| 37 | 2 grams | 1 gram | 5th |
| 61 | 2 grams | 1 gram | 5th |
| 5 | 4 grams | 1 gram | 4th |
| 10 | 6 grams | 200 mg | 4th |
| 19 | 4 grams | 200 mg | 4th |
| 22 | 4 grams | 100 mg | 4th |
| 31 | 4 grams | 500 mg | 4th |
| 37 | 6 grams | 200 mg | 4th |
| 61 | 6 grams | 200 mg | 4th |
| 61 | 4 grams | 250 mg | 4th |
| | Dextrose | | |
| 37 | 2 grams | 1 gram | 5th |
| 49 | 2 grams | 1 gram | 5th |
| 61 | 2 grams | 1 gram | 5th |

The development within this medium was especially rapid, the evolution of gas beginning after the second day and continuing with increased vigor for some time. In the flasks which contained the greater amounts of carbohydrate the evidence of powerful reduction was most pronounced, the surface being rapidly overspread with fusarium and other fungi, which were not apparent on those with lower sugar content. The evolution of gas was so violent in some cases as to force the felt-like growth from the surface, high above the liquid, as illustrated in the accompanying figure. The evidence here presented indicates that the reduction of nitrates is carried on with great vigor in the presence of considerable quantities of carbonaceous material. No appreciable difference could be detected in favor of either mannite or dextrose.

## REDUCTION OF NITRATES CONTINUED

IMPURE CULTURES: The medium used in this experiment was the 1% peptone solution to which was added one gram of potassium nitrate per liter. This solution was distributed in 150 cc. Erlenmeyer flasks in amounts of 50 cc each, and after inoculation with one gram of soil was incubated at 33°C for the length of time and with the results recorded below in table No. VIII.

TABLE VIII

| Soil No. | Time | Per cent of Nitrite |
|---|---|---|
| 1 | 30 hours | 37.50 |
| 2 | 30 hours | 44.00 |
| 3 | 30 hours | 18.75 |
| 4 | 30 hours | 31.25 |
| 5 | 30 hours | 31.25 |
| 6 | 30 hours | 25.00 |
| 7 | 30 hours | 40.00 |
| 8 | 30 hours | 15.00 |
| 9 | 30 hours | 31.25 |
| 10 | 24 hours | 50.00 |
| 11 | 24 hours | 35.00 |
| 12 | 24 hours | 37.50 |
| 13 | 24 hours | 31.30 |
| 14 | 24 hours | 37.50 |
| 15 | 24 hours | 50.00 |
| 16 | 24 hours | 37.50 |
| 17 | 12 hours | 50.00 |
| 18 | 24 hours | 37.50 |
| 19 | 24 hours | 43.80 |
| 20 | 24 hours | 50.00 |
| 21 | 24 hours | 21.88 |
| 22 | 24 hours | 15.00 |
| 23 | 24 hours | 21.88 |
| 24 | 24 hours | 18.75 |
| 25 | 24 hours | 18.75 |
| 26 | 24 hours | 18.75 |
| 27 | 24 hours | 15.25 |
| 28 | 24 hours | 21.88 |
| 29 | 24 hours | 37.50 |
| 30 | 24 hours | 15.25 |
| 31 | 24 hours | 18.75 |
| 32 | 24 hours | 18.75 |

TABLE VIII—Continued

| Soil No. | Time | Per cent of Nitrite |
|---|---|---|
| 33 | 30 hours | 18.75 |
| 34 | 30 hours | 18.75 |
| 35 | 30 hours | 22.00 |
| 36 | 30 hours | 25.00 |
| 37 | 30 hours | 22.50 |
| 38 | 30 hours | 18.75 |
| 39 | 30 hours | 18.75 |
| 40 | 30 hours | 18.75 |
| 41 | 30 hours | 22.50 |
| 42 | 30 hours | 21.87 |
| 43 | 30 hours | 15.63 |
| 44 | 30 hours | 21.87 |
| 45 | 36 hours | 43.75 |
| 46 | 36 hours | 43.75 |
| 47 | 36 hours | 31.25 |
| 48 | 36 hours | 8.75 |
| 49 | 36 hours | 33.30 |
| 50 | 36 hours | 22.50 |
| 51 | 36 hours | 27.50 |
| 52 | 36 hours | 33.30 |
| 53 | 36 hours | .45 |
| 54 | 36 hours | 43.75 |
| 55 | 24 hours | 43.75 |
| 56 | 24 hours | 46.25 |
| 57 | 24 hours | 43.75 |
| 58 | 24 hours | 46.25 |
| 59 | 24 hours | 43.74 |
| 60 | 24 hours | 3.20 |
| 61 | 24 hours | 46.25 |
| 62 | 24 hours | 43.75 |
| 63 | 24 hours | 46.25 |
| 64 | 24 hours | 43.75 |
| 65 | 24 hours | 43.75 |
| 66 | 24 hours | 31.25 |
| 67 | 24 hours | 43.75 |
| 68 | 24 hours | 43.75 |
| 69 | 24 hours | 43.75 |
| 70 | 24 hours | 43.75 |

The following solutions were used for determining the amount of nitrite present:

| | | |
|---|---|---|
| I | a-Naphthylamine........................ | 1.00 gram |
| | Distilled water......................... | 100.00 grams |
| II | Sulphanilic acid........................ | .50 gram |
| | Dilute acetic acid...................... | 150.00 cc |

These solutions were kept in separate glass stoppered bottles.

In the performance of the operation 5 cc of the culture medium were transferred to a Nesslerizing tube by means of a pipette, about 25 cc of distilled water added, and 1 cc of each of the above solutions introduced. The solution was brought up to the 50 or 100 cc mark with distilled water. The quantitative estimation of the nitrite was determined by the colorimic method. Every sample of soil without exception contained bacteria which reduced nitrates to nitrites. The odors emanating from these cultures and from the ammonfication experiments were exceedingly offensive. To determine the possibility of reduction of nitrates in soil infusion, 100 cc of distilled water was measured into each of nine 250 cc Erlenmeyer flasks; to each flask was added 100 mg of potassium nitrate free from nitrite. These flasks were inoculated with soils 5, 47, 49, 51, 54, 55, 57, 60 and 61 respectively. They were incubated at 33°C for seven days. Tests were then made for nitrites and all without exception were found to be negative. No reduction is therefore probable except in the presence of considerable available nitrogen.

## STUDIES ON THE REDUCTION OF NITRITES

IMPURE CULTURES: One hundred cubic centimeters of Ashby's medium without the carbohydrate, were measured into 250 cc Erlenmeyer flasks. To each flask was added varying amounts of mannite, dextrose and potassium salts as indicated. These flasks were inoculated with one gram of the different soils and incubated at 28°C for the length of time and with the results recorded below in table No. IX.

TABLE IX

| Soil No. | Mannite | $KNO_2$ | Day on which Nitrite was Found to be Reduced |
|---|---|---|---|
| 10 | 2 grams | 1 gram | 11 |
| 19 | 2 grams | 1 gram | 11 |
| 22 | 2 grams | 1 gram | 11 |
| 61 | 2 grams | 1 gram | 11 |
| **Soil No.** | **Dextrose** | **$KNO_2$** | |
| 37 | 2 grams | 1 gram | 15 |
| 49 | 2 grams | 1 gram | 15 |
| 61 | 2 grams | 1 gram | 15 |
| 61 | 4 grams | 1 gram | 18 |

Soils number 37, 49, and 61 in dextrose gave on the eleventh day very appreciable reaction for nitrite; not until the fifteenth day did this totally disappear. An inspection of this table conveys the idea at once that the nitrite disappears from the mannite-containing medium more rapidly than from the dextrose.

## THE REDUCTION OF NITRITES IN PEPTONE SOLUTION

IMPURE CULTURES: Fifty cubic centimeters of a medium containing ten grams of peptone, together with one gram of potassium nitrite per liter of distilled water, were measured into 150 cc Erlenmeyer flasks and sterilized. These flasks were each inoculated with one gram of soil and incubated at 33°C. After periods of time as indicated in the table number X, the solutions were tested for the presence of nitrites. On sterilizing peptone-nitrite solution in the Arnold, the liquid assumes a more decided yellow color than the peptone-nitrate solution. A quantitative determination shows that a portion of the nitrite has combined with the peptone, therefore the results are invariably low. All of the soils contained organisms which rapidly reduced nitrites to free nitrogen. The rapidity

of this reaction is very marked. After twenty-four hours the surface of the liquid is covered with foam, and at the end of two days very little nitrite remains. Those cultures in which the evolution of gas was most pronounced, evolved disagreeable odors; while those which developed but slight activity were comparatively odorless. But few species of bacteria reduce nitrites to free nitrogen in straight peptone media. The fluorescens group are of special importance in producing this change. Representatives of this class were isolated from many of these soils and were probably indigenous to all.

TABLE X

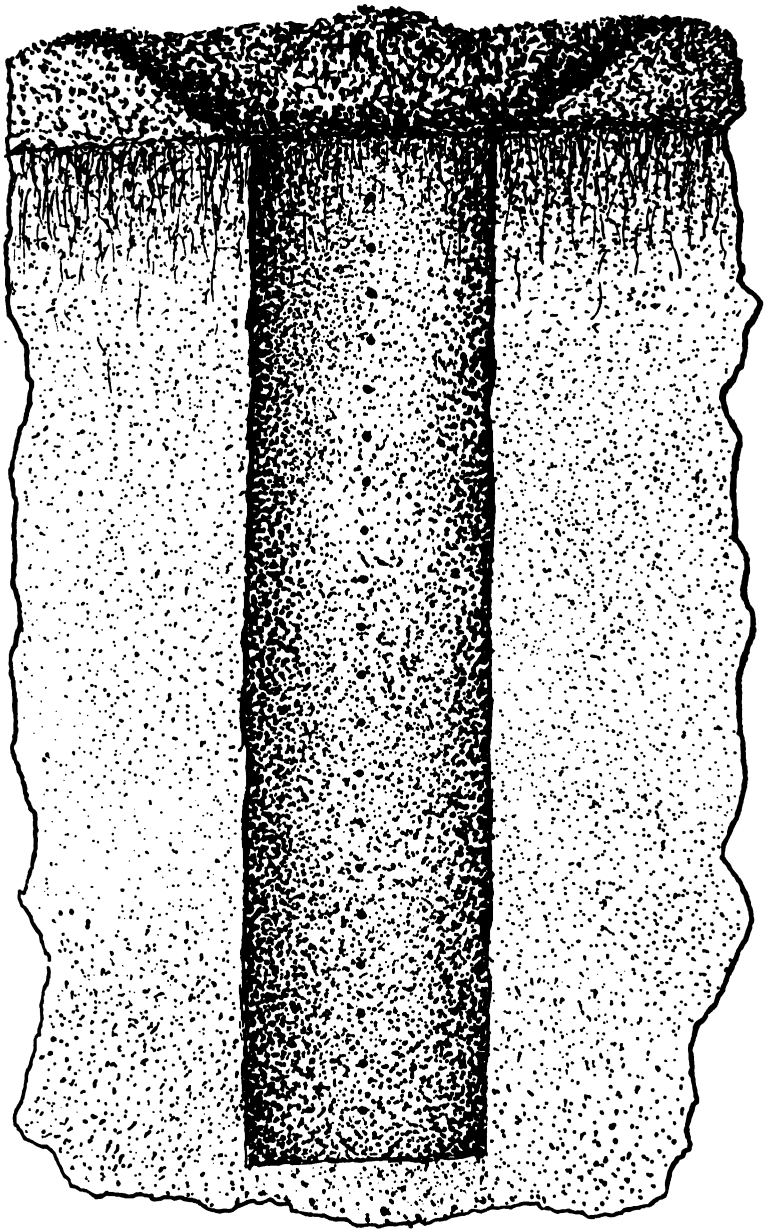
| Soil No. | Time | Per cent of Nitrite |
|---|---|---|
| 1 | 2 days | 28.00 |
| 2 | 2 days | 17.60 |
| 3 | 2 days | 0.00 |
| 4 | 2 days | 14.00 |
| 5 | 2 days | 8.00 |
| 6 | 2 days | 10.00 |
| 7 | 2 days | 14.00 |
| 8 | 2 days | 0.00 |
| 9 | 2 days | 0.00 |
| 10 | 2 days | 12.00 |
| 11 | 2 days | 32.00 |
| 12 | 2 days | 14.00 |
| 13 | 2 days | 50.00 |
| 14 | 2 days | 12.00 |
| 15 | 2 days | 56.00 |
| 16 | 2 days | 24.00 |
| 17 | 2 days | 28.00 |
| 18 | 2 days | 4.00 |
| 19 | 2 days | 56.00 |
| 20 | 2 days | 12.00 |
| 21 | 5 days | 2.40 |
| 22 | 5 days | 0.00 |
| 23 | 5 days | 0.00 |
| 24 | 5 days | 0.00 |
| 25 | 5 days | 5.60 |
| 26 | 5 days | 0.00 |
| 27 | 5 days | 0.00 |
| 28 | 5 days | 0.00 |
| 29 | 5 days | 0.00 |

TABLE X—Continued

| Soil No. | Time | Per cent of Nitrite |
|---|---|---|
| 30 | 5 days | 0.00 |
| 31 | 5 days | 0.00 |
| 32 | 5 days | 0.00 |
| 33 | 6 days | 0.00 |
| 34 | 6 days | 0.00 |
| 35 | 6 days | 0.00 |
| 36 | 6 days | 0.00 |
| 37 | 6 days | 0.00 |
| 38 | 6 days | 0.00 |
| 39 | 6 days | 0.00 |
| 40 | 6 days | 0.00 |
| 41 | 6 days | 0.00 |
| 42 | 6 days | 0.00 |
| 43 | 6 days | 0.00 |
| 44 | 6 days | 0.00 |
| 45 | 3 days | 0.00 |
| 46 | 3 days | 0.00 |
| 47 | 6 days | 0.00 |
| 48 | 6 days | 0.00 |
| 49 | 6 days | 24.00 |
| 50 | 6 days | 0.00 |
| 51 | 6 days | 12.00 |
| 52 | 6 days | 0.00 |
| 53 | 6 days | 0.00 |
| 54 | 4 days | 0.00 |
| 55 | 4 days | 0.00 |
| 56 | 4 days | 0.00 |
| 57 | 4 days | 44.00 |
| 58 | 4 days | 0.00 |
| 59 | 4 days | 0.00 |
| 60 | 4 days | 50.00 |
| 61 | 4 days | 0.00 |
| 62 | 4 days | 70.00 |
| 63 | 4 days | 0.00 |
| 64 | 4 days | 0.00 |
| 65 | 4 days | 0.00 |
| 66 | 4 days | 63.00 |
| 67 | 4 days | 0.00 |
| 68 | 4 days | 0.00 |
| 69 | 4 days | 0.00 |
| 70 | 4 days | 0.00 |

## BACTERIA CONTENT OF THE SUBSOIL

An investigation was begun at the instigation of Hon. George Coupland, Regent of the University of Nebraska, to determine the lowest depth of subsoil in which micro-organisms might be found. In order to facilitate the sampling it was necessary that the subsoil, to the total depth projected, should be exposed. Therefore a hole, which was approximately four feet in diameter and twenty-one feet deep, was dug in an alfalfa field on the farm of Mr. Coupland. Twenty-one samples were taken at intervals of one foot along this perpendicular line. Four surface samples were also taken, east, west, north and south of the excavation, at a distance of ten feet. These samples represent two and four inch depths. All samples were plated both on nutrient agar and on Ashby's medium. Table number XI, shows no striking variation to the sixth foot, except that the fourth level is abnormally high. The oscillation thence to the thirteenth level is neither surprising nor unprecedented, but the great preponderance on the thirteenth level is unaccountable. No visible stratum of impervious earth was observed. Alfalfa roots penetrated to the lowest depth. While the number of bacteria on the thirteenth level was very great, yet, the flora was little diversified. Cladothrix dichotoma being the principal representative. Undoubted azotobacter were isolated from the third level. Of the different species isolated from the above samples, five fermented lactose bouillon with gas production. Fifty-eight per cent reduced nitrates to nitrites.



Excavation showing method of sampling.

## NUMBER OF BACTERIA PER GRAM OF SUBSOIL DRIED TO CONSTANT WEIGHT AT 100—110°C

TABLE XI

| Depth | Nutrient Agar | Ashby's Medium |
|---|---|---|
| 2 inches | 2,500.000 | 610.000 |
| 4 inches | 660,000 | 458,000 |
| 1 foot | 290,000 | 417,000 |
| 2 feet | 282,000 | 250,000 |
| 3 feet | 169,000 | 185,000 |
| 4 feet | 277,000 | 210,000 |
| 5 feet | 156,000 | 114,000 |
| 6 feet | 66,000 | 47,000 |
| 7 feet | 11,000 | 2,000 |
| 8 feet | 7.400 | 7,100 |
| 9 feet | 700 | 300 |
| 10 feet | 1,200 | 1,000 |
| 11 feet | 4,700 | 2,700 |
| 12 feet | 1,200 | 2,600 |
| 13 feet | 26,500 | 116,000 |
| 14 feet | 50 | 000 |
| 15 feet | 000 | 000 |
| 16 feet | 000 | 000 |
| 18 feet | 000 | 000 |
| 19 feet | 000 | 000 |
| 20 feet | 000 | 000 |

## THE FATE OF UREA IN THE SOIL

IMPURE CULTURES: To asertain the changes which take place when urea is added to nitrogenous media, 100 cc of the 1% peptone solution were measured into 250 cc Erlenmeyer flasks and sterilized. To each flask was added one gram of urea. They were then inoculated with soils 1, 10, 13, 25, 27, 28, 34, 35, 45, 49, 61 and 68. After seven days incubation at 33°C, each flask gave off strong odor of ammonia. An inquiry into the presence of carbonate was then instituted with positive result. I therefore conclude that the organisms which transform urea to ammonium carbonate in the presence of abundant available nitrogen supply, are universally distributed within our soil. To ascertain the trend of the reaction

in nitrogen-poor media, flasks were filled with Ashby's medium as before, one gram of urea introduced, and inoculated as in the nitrogenous medium. After forty eight hours the surface was covered with gas bubbles and on the fourth day a strong odor of ammonia was evolved from each flask. It therefore appears that in the presence of an abundant nitrogen supply urea is converted into ammonium carbonate, and that this process is not impeded by the presence of carbohydrate in great excess, but is rather promoted, even though the nitrogen content be very small.

Several flasks of Ashby's medium were inoculated with soils and thio-urea introduced in place of urea. The growth in these flasks was much less pronounced than in those containing urea. Evidently ammonium sulphite was not formed.

Hippuric acid is split, in nitrogenous media, into benzoic acid and amino-acetic acid. Several flasks of Ashby's medium were inoculated with soil and one gram of hippuric acid introduced. After a few days the surface wás overgrown with molds, later a vigorous evolution of carbon dioxide was perceptible, the overlying growth being forced high in the flask. In a second experiment the hippuric acid was neutralized with sodium hydroxide before being transferred to the Ashby's medium. The splitting of the hippuric acid molecule into benzoic acid and amino-acetic acid, and the subsequent union of the benzoic acid and calcium carbonate to form calcium benzoate, necessitates the liberation of considerable quantities of carbon dioxide. Amino-acetic acid (Glycocoll) is reduced by soil bacteria to ammonia and acetic acid, this reduction is consummated both in nitrogenous and non-nitrogenous media.

## THE REDUCTION OF NITRATES TO NITRITES

The breaking down of organic compounds by bacterial agency, falls under two categories; simple cleavage, and partial elementary disintegration of the proteid and carbohydrate molecule. In the first category we are concerned with the simple splitting off of groups from the original relatively complex molecule. Among the cleavage products may be mentioned alcohols, esters, mercaptans, amino-acids, phenol,

skatol, indol, acids, glycols, etc. The formation of nascent hydrogen by the action of destructive organisms on carbohydrate and proteid compounds may be best illustrated by a careful study of the products obtained by the destructive distillation of coal, wood and other products of animal and vegetable origin. In the destructive distillation of coal we get as products: $O_2$, $H_2$, $N_2$, $S_2$, Cx, $H_2O$, $NH_3$, $H_2S$, $CH_4$, CO, $CO_2$, $C_2H_2$, $C_2H_4$, $C_6H_6$, $CS_2$, etc. All of these products are obtained in small or large amounts depending on the composition of the coal, character of heating, etc. Can these products be explained in any other way than that the complex proteid molecules undergo in this process of destructive distillation, complete disintegration into their constitutent elements: C, O, H, N, S.? These elements must exist momentarily in the active or nascent state. Because of their great chemical affinity these active elements then combine with each other to form inactive molecules which are free to pass off from the sphere of action. In the formation of these simple molecules some of the atoms have combined with different atoms, while some have combined with other atoms of the same kind. As a result of the first method we get: $H_2O$, $NH_3$, $H_2S$, CO, $CO_2$, $C_2H_4$, $CH_4$, etc. As a result of the second method we get: Cx (coke or soot), $N_2$, $H_2$, $O_2$, $S_2$, etc.

For the reduction of nitrates in pure culture, a medium consisting of peptone 1% and potassium nitrate 1% was employed. 10 cc of this medium were introduced into each tube and sterilized. These tubes were inoculated with the various organisms and incubated at 33°C for ten days. At the expiration of this time they were tested for the presence of nitrates and nitrites. The presence of nitrate was determined by the addition of a 1% solution of diphenylamine in pure concentrated sulphuric acid. The nitrite was determined according to the method used in the previous work on impure cultures. Those cultures which failed to reduce nitrates to nitrites within ten days were duplicated and the time extended to thirty days for a final reading.

The organisms in the following list were obtained from the celebrated Kral collection, Vienna Austria, Prof. Kraus Curator; and from the American Museum of Natural History,

New York, Prof. C. E. A. Winslow Curator. No attempt was made to determine whether they were true to name, the only precaution being that they were in pure culture. It is quite improbable that any considerable collection of species would be assembled without some repetition under different names. Not all the organisms listed are strictly soil bacteria, several of the intestinal group being purposely included. A few have no connection with soil fertility whatever.

## CATALOG OF ORGANISMS

1. Bacterium acetosum [*Henneberg*]
2. Bacterium lactis aerogenes [*Escherich*]
3. Bacillus brassicae acidae
4. Micrococcus agilis [*Ali-Cohen*]
5. Bacillus acidi lactici [*Hueppe*]
6. Micrococcus albidus
7. Bacillus amylovorus
8. Bacillus anthracis
9. Bacillus pseudo-anthracis
10. Bacillus anthracoides
11. Bacterium annulatum A
12. Bacterium annulatum B
13. Bacillus aquatile
14. Bacillus arborescens [*Frankland*]
15. Bacillus argentinensis [*Kayser*]
16. Micrococcus ascoformans
17. Bacillus asterosporus
18. Bacterium aurantiacus
19. Sarcina aurantiaca
20. Bacillus Baccarinii [*Macchiati*]
21. Bacterium beticolum
22. Micrococcus brunneus
23. Bacillus budapestinensis [*Ajtay*]
24. Bacillus butyricus [*Hueppe*]
25. Bacillus candicans
26. Micrococcus candicans [*Flugge*]
27. Monila candida
28. Bacillus campestris
29. Rhodobacillus capsulatus

30. Bacillus cereus [*Frankland*]
31. Bacillus cereulens
32. Micrococcus cereus
33. Micrococcus carneus
34. Micrococcus cinnabareus
35. Bacillus cloacae [*Jordan*]
36. Micrococcus citreus
37. Bacillus constrictus
38. Micrococcus concentricus
39. Bacillus coli commune [*Kruse*]
40. Bacillus coli-anaerogenes
41. Bacillus carotovorus [*Jones*]
42. Bacillus cyanogenes [*Hueppe*]
43. Bacillus cylindrosporus [*Burchard*]
44. Bacillus creusus
45. Bacillus cyaneus
46. Bacterium crysogloia
47. Bacillus denitrificans
48. Bacillus dendroides
49. Pseudomonas destructans
50. Bacillus disciformans
51. Bacillus enteritidis [*Gaertner*]
52. Bacillus esterigenes [*Kral*]
53. Bacillus esterigenes A
54. Bacillus esterigenes D
55. Bacterium lactis erythrogenes [*Grotenfeldt*]
56. Bacillus ethacinicus
57. Bacillus ethaceto succinicus
58. Bacillus ferruginous
59. Bacillus faecalis alcaligenes [*Petruschky*]
60. Sarcina flava
61. Micrococcus flavus [*Flugge*]
62. Bacillus flavidus
63. Bacterium filiforme [*Henrici*]
64. Bacterium filifaciens [*H. Jensen*]
65. Bacillus Fitzianus
66. Bacillus fluorescens liquefaciens [*Flugge*]
67. Bacillus fluorescens non liquefaciens
68. Bacillus fluorescens tenuis
69. Bacillus Frostii

70. Bacillus fuchsinus [*Balkhout*]
71. Sarcina gasformans
72. Bacillus graviolens [*A. Meyer et Gottheil*]
73. Bacterium aquatile griseum
74. Micrococcus grossus [*Henrici*]
75. Bacterium Hartlebi [*H. Jensen*]
76. Bacillus havaniensis
77. Bacillus herbicoli aureus
78. Bacillus helvolus [*Zimmermann*]
79. Bacillus hoagii
80. Bacillus hyponitrous
81. Bacillus immobile
82. Bacillus indicus
83. Bacillus indigoferus [*Voges*]
84. Bacillus irritans
85. Bacillus ivilans
86. Bacillus jasminocyaneus
87. Bacillus juglandis
88. Bacillus kiliensis
89. Bacillus lactis
90. Bacillus lactorubefaciens [*Gruber*]
91. Bacillus lateritia
92. Bacillus levans
93. Bacillus lactis amari liquefaciens [*Freudenreich*]
94. Bacillus liodermos
95. Bacillus limosus
96. Sarcina liquefaciens [*Frankland*]
97. Bacillus liquefaciens
98. Bacillus lactis niger
99. Bacillus liquefaciens niger
100. Bacillus loxosus [*Burchard*]
101. Bacterium aquatile gasformans non liquefaciens
102. Micrococcus luteus
103. Sarcina lutea
104. Streptococcus luteus liquefaciens
105. Bacillus maidis
106. Bacillus melonis [*Winslow*]
107. Bacillus mesentericus fuscus
108. Bacillus mesentericus niger
109. Bacillus mesentericus ruber

110. Bacillus mesentericus vulgatus
111. Bacillus megatherium [*De Bary*]
112. Bacillus miniaceus [*Zimmermann*]
113. Bacillus proteus mirabilis
114. Sarcina mobilis
115. Moellers grass bacillus, Mist.
116. Bacterium muris [*E. Klein*]
117. Bacillus mycoides [*Flugge*]
118. Bacillus nanus
119. Bacillus ochraceus [*Zimmermann*]
120. Bacillus oleraceae
121. Bacillus olfactorius
122. Oidium lactis
123. Bacillus oleae [*Schiff-Giorgini*]
124. Cladothrix odorifera
125. Cladothrix dichotoma.
126. Bacillus oxalatus
127. Bacterium para-coli gasformans anindolicum [*Kayser*]
128. Bacillus parvus
129. Rhodobacillus palustis
130. Bacillus Petasites [*A. Meyer et Gottheil*]
131. Bacterium Petroselini [*Burchard*]
132. Bacillus prodigiosus [*Flugge*]
133. Bacillus lactis proteolyticus [*Rullman*]
134. Bacillus plicatus
135. Bacterium phytophtorum
136. Bacillus proteus
137. Bacillus pumilis [*A. Meyer et Gottheil*]
138. Bacillus punctatus
139. Bacillus fluorescens putidus [*Flugge*]
140. Bacillus phosphorescens
141. Pseudomonas pyocyanea
142. Bacterium radiatum [*Kayser*]
143. Pseudomonas radicicola, clover
144. Bacillus ramosus non liquefaciens
145. Bacillus rosaceus
146. Micrococcus roseus [*Eisenberg*]
147. Bacillus of ropy milk
148. Micrococcus rhodochrous
149. Bacillus brunneus mycoides roseus

150. Bacillus capsulatus roseus
151. Bacillus ruber
152. Micrococcus ruber
153. Bacillus subtilis var ruber
154. Bacillus ruber Plymouth
155. Bacillus rubidus
156. Spirillum rubrum
157. Bacterium rugosum [*Henrici*]
158. Bacillus ruber of Kiel
159. Spirillum rugula
160. Bacterium rubilum
161. Bacillus ruminatus
162. Bacillus rutilus
163. Bacillus rutilensis
164. Spirillum serpens
165. Bacillus silvaticus [*Arthur Meyer et Neide*]
166. Bacillus simplex [*A. Meyer et Gottheil*]
167. Vibrio saprophilus
168. Micrococcus sordidus
169. Bacillus luteus sporogenes [*Wood, Smith et Baker*]
170. Bacterium der sorbose
171. Bacillus solanisaprus
172. Bacillus sphaericus [*Arthur, Meyer et Neide*]
173. Staphlococcus cereus aureus
174. Staphlococcus pyogenes citreus
175. Staphlococcus pyogenes albus
176. Staphlococcus pyogenes aureus
177. Bacillus ochraceus subflavus
178. Bacterium subflavum
179. Micrococcus sulfur
180. Bacillus subtilis [*Ehrenberg*]
181. Bacterium Stutzeri [*H. Jensen*]
182. Bacillus synxanthus [*Cohn*]
183. Bacterium tremellioides [*Schottelius*]
184. Bacillus tumefaciens
185. Bacillus tumescens
186. Bacillus typhosus [*Eberth*]
187. Bacillus para-typhosus
188. Sarcina ventriculi [*Goodsir*]
189. Bacillus violaceus [*Jordan*]

190. Azotobacter vinelandii [*Lipman*]
191. Micrococcus viticulosus
192. Bacillus proteus viridis
193. Bacillus aquatile villos
194. Bacillus vivax
195. Spirillum volutans
196. Bacillus proteus vulgaris [*Hauser*]
197. Bacterium xanthochlorum
198. Bacillus xylinum
199. Bacillus proteus Zenkeri [*Hauser*]
200. Bacillus Zopfii
201. Boden I. [*Tsiklinsky-Sudpolarexpedition 1903–5*]

1. Bacterium acetosum: good growth; vigorous evolution of gas on addition of acid; nitrate content considerably diminished;
Strong formation of nitrite.
2. Bacterium lactis aerogenes: good growth; vigorous evolution of gas on addition of acid; nitrate content greatly diminished;
Strong formation of nitrite.
3. Bacillus brassicae acidae: good growth; moderate evolution of gas on addition of acid; nitrate content considerably diminished;
Weak formation of nitrite.
4. Micrococcus agilis: good growth; no evolution of gas on addition of acid; nitrate content unchanged;
No formation of nitrite.
5. Bacillus acidi lactici: good growth; vigorous evolution of gas on addition of acid; nitrate content greatly diminished;
Strong formation of nitrite.
6. Micrococcus albidus: moderate growth; slight evolution of gas on addition of acid; nitrate content not greatly diminished;
Weak formation of nitrite.
7. Bacillus amylovorus: good growth; strong evolution of gas on addition of acid; nitrate content greatly diminished;
Strong formation of nitrite.
8. Bacillus anthracis: good growth; vigorous evolution of gas on addition of acid; nitrate content greatly diminished;
Strong formation on nitrite.
9. Bacillus pseudo-anthracis: good growth; no evolution of gas on addition of acid; nitrate content unchanged;
No formation of nitrite.
10. Bacillus anthracoides: good growth; moderate evolution of gas on addition of acid; nitrate content slightly diminished;
Weak formation of nitrite.

11. Bacterium annulatum A: good growth; vigorous evolution of gas on addition of acid; nitrate content greatly diminished;
Strong formation of nitrite.
12. Bacterium annulatum B: good growth; vigorous evolution of gas on addition of acid; nitrate content greatly diminished;
Strong formation of nitrite.
13. Bacillus aquatile: good growth; vigorous evolution of gas on addition of acid; nitrate content greatly diminished;
Strong formation of nitrite.
14. Bacillus arborescens: good growth; vigorous evolution of gas addition of acid; nitrate content greatly diminished;
Strong formation of nitrite.
15. Bacillus argentinensis: good growth; moderate evolution of gas on addition of acid; nitrate content slightly diminished;
Weak formation of nitrite.
16. Micrococcus ascoformans: fair growth; slight evolution of gas on addition of acid; nitrate content not greatly diminished;
Weak formation of nitrite.
17. Bacillus asterosporus: scant growth; slight evolution of gas on addition of acid; nitrate content not greatly diminished;
Weak formation of nitrite.
18. Bacterium aurantiacus: good growth; no evolution of gas on addition of acid; nitrate content unchanged;
No formation of nitrite.
19. Sarcina aurantiaca: scant growth; no evolution of gas on addition of acid; nitrate content unchanged;
No formation of nitrite.
20. Bacillus Baccarinii: fair growth; slight evolution of gas on addition of acid; nitrate content slightly diminished;
Weak formation of nitrite.
21. Bacterium beticolum: good growth; vigorous evolution of gas on addition of acid; nitrate content greatly reduced;
Moderate formation of nitrite.
22. Micrococcus brunneus: moderate growth; slight evolution of gas on addition of acid; nitrate content not greatly diminished;
Weak formation of nitrite.
23. Bacillus budapestiensis: good growth; no evolution of gas on addition of acid; nitrate content very slightly diminished;
Very weak formation of nitrite.
24. Bacillus butyricus: good growth; no evolution of gas on addition of acid; nitrate content unchanged'
No formation of nitrite.
25. Bacillus candicans: moderate growth; no evolution of gas on addition of acid; nitrate content unchanged;
No formation of nitrite.
26. Micrococcus candicans: moderate growth; no evolution of gas on addition of acid; nitrate content unchanged;
No formation of nitrite.

27. Monila candida: fair growth; slight evolution of gas on addition of acid; nitrate content not greatly diminished;
Weak formation of nitrite.
28. Bacillus campestris: good growth; no evolution of gas on addition of acid; nitrate content unchanged;
No formation of nitrite.
29. Rhodobacillus capsulatus: moderate growth; no evolution of gas on addition of acid; nitrate content unchanged;
No formation of nitrite.
30. Bacillus cereus: good growth; vigorous evolution of gas on addition of acid; nitrate content greatly diminished;
Strong formation of nitrite.
31. Bacillus cereulens: good growth; moderate evolution of gas on addition of acid; nitrate content appreciably diminished;
Weak formation of nitrite.
32. Micrococcus cereus: moderate growth; no evolution of gas on addition of acid; nitrate content unchanged;
No formation of nitrite.
33. Micrococcus carneus: good growth; no evolution of gas on addition of acid; nitrate content unchanged;
No formation of nitrite.
34. Micrococcus cinnabareus: fair growth; slight evolution of gas on addition of acid; nitrate content not greatly diminished;
Weak formation of nitrite.
35. Bacillus cloacae: good growth; vigorous evolution of gas on addition of acid; nitrate content greatly diminished;
Strong formation of nitrite.
36. Micrococcus citreus: fair growth; no evolution of gas on addition of acid; nitrate content unchanged;
No formation of nitrite.
37. Bacillus constrictus: good growth; vigorous evolution of gas on addition of acid nitrate content greatly diminished;
Strong formation of nitrite.
38. Micrococcus concentricus: fair growth; no evolution of gas on addition of acid; nitrate content unchanged;
No formation of nitrite.
39. Bacillus coli commune: good growth; vigorous evolution of gas on addition of acid; nitrate content greatly diminished;
Strong formation of nitrite.
40. Bacillus coli-anaerogenes: good growth; vigorous evolution of gas on addition of acid; nitrate content greatly diminished;
Strong formation of nitrite.
41. Bacillus carotovorus: fair growth; slight evolution of gas on addition of acid; nitrate content not greatly diminished;
Weak formation of nitrite.
42. Bacillus cyanogenes: good growth; slight evolution of gas on addition of acid; nitrate content not greatly diminished;
Weak formation of nitrite.

43. Bacillus cylindrosporus: fair growth; slight evolution of gas on addition of acid; nitrate content not greatly diminished;
Weak formation of nitrite.
44. Bacillus creusus: good growth; moderate evolution of gas on addition of acid; nitrate content considerably diminished;
Moderate formation of nitrite.
45. Bacillus cyaneus: good growth; no evolution of gas on addition of acid; nitrate content unchanged;
No formation of nitrite.
46. Bacterium crysogloia: moderate growth; no evolution of gas on addition of acid; nitrate content unchanged;
No formation of nitrite.
47. Bacillus denitrificans: good growth; vigorous evolution of gas on addition of acid; nitrate content greatly diminished;
Strong formation of nitrite.
48. Bacillus dendroides: fair growth; no evolution of gas on addition of acid; nitrate content unchanged;
No formation of nitrite.
49. Pseudomonas destructans: moderate growth; fair evolution of gas on addition of acid; nitrogen content slightly diminished;
Weak formation of nitrite.
50. Bacillus disciformans: good growth; vigorous evolution of gas on addition of acid; nitrate content greatly diminished;
Strong formation of nitrite.
51. Bacillus enteritidis: good growth; vigorous evolution of gas on addition of acid; nitrate content greatly diminished;
Strong formation of nitrite.
52. Bacillus esterigenes: fair growth; no evolution of gas on addition of acid; nitrate content unchanged;
No formation of nitrite.
53. Bacillus esterigenes A: good growth; no evolution of gas on addition of acid; nitrate content unchanged;
No formation of nitrite.
54. Bacillus esterigenes D: good growth; no evolution of gas on addition of acid; nitrate content unchanged;
No formation of nitrite.
55. Bacterium lactis erythrogenes: good growth; slight evolution of gas on addition of acid; nitrate content not greatly diminished;
Weak formation of nitrite.
56. Bacillus ethacinicus: fair growth; moderate evolution of gas on addition of acid; nitrate content slightly diminished;
Weak formation of nitrite.
57. Bacillus ethaceto succinicus: good growth; moderate evolution of gas on addition of acid; nitrate content slightly diminished;
Weak formation of nitrite.
58. Bacillus ferruginous: good growth; vigorous evolution of gas on addition of acid; nitrate content greatly diminished;
Strong formation of nitrite.

59. Bacillus faecalis alcaligenes: good growth; slight evolution of gas on addition of acid; nitrate content not greatly diminished;
Weak formation of nitrite.

60. Sarcina flava: fair growth; slight evolution of gas on addition of acid; nitrate content not greatly diminished;
Weak formation of nitrite.

61. Micrococcus flavus: moderate growth; slight evolution of gas on addition of acid; nitrate content not greatly diminished;
Weak formation of nitrite.

62. Bacillus flavidus: moderate growth; weak evolution of gas on addition of acid; nitrate content not greatly diminished;
Weak formation of nitrite.

63. Bacterium filiforme: good growth; slight evolution of gas on addition of acid; nitrate content not greatly diminished;
Weak formation of nitrite.

64. Bacterium filifaciens: good growth; vigorous evolution of gas on addition of acid; nitrate content greatly diminished;
Strong formation of nitrite.

65. Bacillus Fitzianus: good growth; slight evolution of gas on addition of acid; nitrate content not greatly diminished;
Weak formation of nitrite.

66. Bacillus fluorescens liquefaciens: good growth; vigorous evolution of gas on addition of acid; nitrate content greatly diminished;
Strong formation of nitrite.

67. Bacillus fluorescens non-liquefaciens: good growth; vigorous evolution of gas on addition of acid; nitrate content greatly diminished;
Strong formation of nitrite.

68. Bacillus fluorescens tenuis: good growth; no evolution of gas on addition of acid; nitrate content unchanged;
No formation of nitrite.

69. Bacillus Frostii: good growth; slight evolution of gas on addition of acid; nitrate content not greatly diminished.
Weak formation of nitrite.

70. Bacillus fuchsinus: moderate growth; no evolution of gas on addition of acid; nitrate content unchanged;
No formation of nitrite.

71. Sarcina gasformans: moderate growth; no evolution of gas on addition of acid; nitrite content unchanged;
No formation of nitrite.

72. Bacillus graviolens: good growth; no evolution of gas on addition of acid; nitrate content unchanged;
No formation of nitrite.

73. Bacterium aquatile griseum: good growth; vigorous evolution of gas on addition of acid; nitrate content greatly diminished;
Strong formation of nitrite.

74. Micrococcus grossus: moderate growth; slight evolution of gas on addition of acid; nitrate content not greatly diminished;
Weak formation of nitrite.

**75.** **Bacterium Hartlebi:** good growth; vigorous evolution of gas on addition of acid; nitrate content greatly diminished;
Strong formation of nitrite.

**76.** **Bacillus Havaniensis:** good growth; moderate evolution of gas on addition of acid; nitrate content slightly diminished;
Weak formation of nitrite.

**77.** **Bacillus herbicoli aureus:** moderate growth; slight evolution of gas on addition of acid; nitrate content diminished;
Weak formation of nitrite.

**78.** **Bacillus helvolus:** good growth; moderate evolution of gas on addition of acid; nitrate content diminished;
Fair formation of nitrite.

**79.** **Bacillus Hoagii:** moderate growth; fair evolution of gas on addition of acid; nitrate content slightly diminished;
Weak formation of nitrite.

**80.** **Bacillus hyponitrous:** scant growth; no evolution of gas on addition of acid; nitrate content unchanged;
No formation of nitrite.

**81.** **Bacillus immobile:** good growth; vigorous evolution of gas on addition of acid; nitrate content greatly diminished;
Strong formation of nitrite.

**82.** **Bacillus indicus:** good growth; slight evolution of gas on addition of acid; nitrate content not greatly diminished;
Weak formation of nitrite.

**83.** **Bacillus indigoferus:** good growth; moderate evolution of gas on addition of acid; nitrate content slightly diminished;
Moderate formation of nitrite.

**84.** **Bacillus irritans:** good growth; moderate evolution of gas on addition of acid; nitrate content slightly diminished;
Weak formation of nitrite.

**85.** **Bacillus ivilans:** moderate growth; slight evolution of gas on addition of acid; nitrate content not greatly diminished;
Weak formation of nitrite.

**86.** **Bacillus jasminocyaneus:** good growth; vigorous evolution of gas on addition of acid; nitrate content greatly diminished;
Strong formation of nitrite.

**87.** **Bacillus juglandis:** good growth; no evolution of gas on addition of acid; nitrate content unchanged;
No formation of nitrite.

**88.** **Bacillus kiliensis:** moderate growth; no evolution of gas on addition of acid; nitrate content unchanged;
No formation of nitrite.

**89.** **Bacillus lactis:** good growth; vigorous evolution of gas on addition of acid; nitrate content greatly diminished;
Strong formation of nitrite.

**90.** **Bacillus lactorubefaciens:** good growth; vigorous evolution of gas on addition of acid; nitrate content greatly diminished;
Strong formation of nitrite.

91. Bacillus lateritia: good growth; slight evolution of gas on addition of acid; nitrate content almost unchanged;
Very weak formation of nitrite.

92. Bacillus levans: moderate growth; no evolution of gas on addition of acid; nitrate content unchanged;
No formation of nitrite.

93. Bacillus lactis amari liquefaciens: good growth; slight evolution of gas on addition of acid; nitrate content slightly diminished;
Weak formation of nitrite.

94. Bacillus liodermos: good growth; moderate evolution of gas on addition of acid; nitrate content considerably diminished;
Moderate formation of nitrite.

95. Bacillus limosus: moderate growth; slight evolution of gas on addition of acid; nitrate content not materially changed;
Weak formation of nitrite.

96. Sarcina liquefaciens: fair growth; no evolution of gas on addition of acid; nitrate content unchanged;
No formation of nitrite.

97. Bacillus liquefaciens: good growth; no evolution of gas on addition of acid; nitrate content unchanged;
No formation of nitrite.

98. Bacillus lactis niger: good growth; moderate evolution of gas on addition of acid; nitrate content not greatly diminished;
Weak formation of nitrite.

99. Bacillus liquefaciens niger: good growth; moderate evolution of gas on addition of acid; nitrate content slightly diminished;
Weak formation of nitrite.

100. Bacillus loxosus: good growth; vigorous evolution of gas on addition of acid; nitrate content greatly diminished;
Strong formation of nitrite.

101. Bacterium aquatile gasformans non-liquefaciens: good growth; vigorous evolution of gas on addition of acid; nitrate content greatly diminished;
Strong formation of nitrite.

102. Micrococcus luteus: fair growth; no evolution of gas on addition of acid; nitrate content unchanged;
No formation of nitrite.

103. Sarcina lutea: moderate growth; no evolution of gas on addition of acid; nitrate content unchanged;
No formation of nitrite.

104. Streptococcus luteus liquefaciens: good growth; no evolution of gas on addition of acid; nitrate content unchanged;
No formation of nitrite.

105. Bacillus maidis: scant growth; no evolution of gas on addition of acid; nitrate content unchanged;
No formation of nitrite.

106. Bacillus melonis: good growth; vigorous evolution of gas on addition of acid; nitrate content greatly diminished;
Strong formation of nitrite.

107. Bacillus mesentericus fuscus: good growth; vigorous evolution of gas on addition of acid; nitrate content greatly diminished;
Strong formation of nitrite.

108. Bacillus mesentericus niger: good growth; vigorous evolution of gas on addition of acid; nitrate content greatly diminished;
Strong formation of nitrite.

109. Bacillus mesentericus ruber: good growth; vigorous evolution of gas on addition of acid; nitrate content greatly diminished;
Strong formation of nitrite.

110. Bacillus mesentericus vulgatus: good growth; slight evolution of gas on addition of acid; nitrate content slightly diminished;
Weak formation of nitrite.

111. Bacillus megatherium: good growth; no evolution of gas on addition of acid; nitrate content unchanged;
No formation of nitrite.

112. Bacillus miniaceus: good growth; vigorous evolution of gas on addition of acid; nitrate content greatly diminished;
Strong formation of nitrite.

113. Bacillus proteus mirabilis: good growth; no evolution of gas on addition of acid; nitrate content unchanged;
No formation of nitrite.

114. Sarcina mobilis: fair growth; slight evolution of gas on addition of acid; nitrate content slightly diminished;
Weak formation of nitrite.

115. Moeller's grass bacillus, Mist: good growth; moderate evolution of gas on addition of acid; nitrate content slightly diminished;
Moderate formation of nitrite.

116. Bacterium muris: fair growth; no evolution of gas on addition of acid; nitrate content unchanged;
No formation of nitrite.

117. Bacillus mycoides: good growth; vigorous evolution of gas on addition of acid; nitrate content greatly diminished;
Strong formation of nitrite.

118. Bacillus nanus: good growth; vigorous evolution of gas on addition of acid; nitrate content greatly diminished;
Strong formation of nitrite.

119. Bacillus ochraceus: good growth; no evolution of gas on addition of acid; nitrate content unchanged;
No formation of nitrite.

120. Bacillus oleraceae: moderate growth; fair evolution of gas on addition of acid; nitrate content slightly diminished;
Weak formation of nitrite.

121. Bacillus olfactorius: good growth; vigorous evolution of gas on addition of acid; nitrate content greatly diminished;
Strong formation of nitrite.

122. Oidium lactis: good growth; no evolution of gas on addition of acid; nitrate content unchanged;
No formation of nitrite.

123. Bacillus oleae: good growth; vigorous evolution of gas on addition of acid; nitrate content greatly diminished;
Strong formation of nitrite.

124. Cladothrix odorifera: good growth; no evolution of gas on addition of acid; nitrate content unchanged;
No formation of nitrite.

125. Cladothrix dichotoma: good growth; no evolution of gas on addition of acid; nitrate content unchanged;
No formation of nitrite.

126. Bacillus oxalatus: good growth; strong evolution of gas on addition of acid; nitrate content greatly diminished;
Strong formation of nitrite.

127. Bacterium paracoli gasformans anindolicum: good growth; moderate evolution of gas on addition of acid; nitrate content slightly diminished;
Moderate formation of nitrite.

128. Bacillus parvus: good growth; vigorous evolution of gas on addition of acid; nitrate content greatly diminished;
Strong formation of nitrite.

129. Rhodobacillus palustis: good growth; vigorous evolution of gas on addition of acid; nitrate content greatly diminished;
Strong formation of nitrite.

130. Bacillus Petasites: good growth; no evolution of gas on addition of acid; nitrate content unchanged;
No formation of nitrite.

131. Bacterium Petroselini: good growth; moderate evolution of gas on addition of acid; nitrate content slightly diminished;
Weak formation of nitrite.

132. Bacillus prodigiosus: good growth; vigorous evolution of gas on addition of acid; nitrate content greatly diminished;
Strong formation of nitrite.

133. Bacillus lactis proteolyticus: good growth; vigorous evolution of gas on addition of acid; nitrate content greatly diminished;
Strong formation of nitrite.

134. Bacillus plicatus: moderate growth; slight evolution of gas on addition of acid; nitrate content slightly diminished;
Weak formation of nitrite.

135. Bacterium phytophtorum: fair growth; no evolution of gas on addition of acid; nitrate content unchanged;
No formation of nitrite.

136. Bacillus proteus: good growth; moderate evolution of gas on addition of acid; nitrate content slightly diminished;
Weak formation of nitrite.

137. Bacillus pumilus: good growth; no evolution of gas on addition of acid; nitrate content unchanged;
No formation of nitrite.

138. Bacillus punctatus: good growth; vigorous evolution of gas on addition of acid; nitrate content greatly diminished;
Strong formation of nitrite.

139. Bacillus fluorescens putidus: moderate growth; no evolution of gas on addition of acid; nitrate content undiminished;
No formation of nitrite.

140. Bacillus phosphorescens: good growth; vigorous evolution of gas on addition of acid; nitrate content greatly diminished;
Strong formation of nitrite.

141. Pseudomonas pyocyanea: good growth; vigorous evolution of gas on addition of acid; nitrate content greatly diminished;
Strong formation of nitrite.

142. Bacterium radiatum: good growth; no evolution of gas on addition of acid; nitrate content unchanged;
No formation of nitrite.

143. Pseudomonas radicicola, clover: good growth; vigorous evolution of gas on addition of acid; nitrate content greatly diminished;
Strong formation of nitrite.

144. Bacillus ramosus non liquefaciens: good growth; no evolution of gas on addition of acid; nitrate content unchanged;
No formation of nitrite.

145. Bacillus rosaceus: good growth; no evolution of gas on addition of acid; nitrate content unchanged;
No formation of nitrite.

146. Micrococcus roseus: good growth; vigorous evolution of gas on addition of acid; nitrate content greatly diminished;
Strong formation of nitrite.

147. Bacillus of ropy milk: good growth; vigorous evolution of gas on addition of acid; nitrate content greatly diminished;
Strong formation of nitrite.

148. Micrococcus rhodochrous: moderate growth; slight evolution of gas on addition of acid; nitrate content not greatly diminished;
Weak formation of nitrite.

149. Bacillus brunneus mycoides roseus: good growth; vigorous evolution of gas on addition of acid; nitrate content greatly diminished;
Strong formation of nitrite.

150. Bacillus capsulatus roseus: good growth; vigorous evolution of gas on addition of acid; nitrate content greatly diminished;
Strong formation of nitrite.

151. Bacillus ruber: moderate growth; fair evolution of gas on addition of acid; nitrate content slightly diminished;
Weak formation of nitrite.

152. Micrococcus ruber: moderate growth; fair evolution of gas on addition of acid; nitrate content slightly diminished;
Weak formation of nitrite.

153. Bacillus subtilis var ruber: good growth; vigorous evolution of gas on addition of acid; nitrate content greatly diminished;
Strong formation of nitrite.

154. Bacillus ruber Plymouth: good growth; vigorous evolution of gas on addition of acid; nitrate content greatly diminished;
Strong formation of nitrite.

155. Bacillus rubidus: good growth; no evolution of gas on addition of acid; nitrate content unchanged;
No formation of nitrite.

156. Spirillum rubrum: moderate growth; no evolution of gas on addition of acid; nitrate content unchanged;
No formation of nitrite.

157. Bacterium rugosum: good growth; vigorous evolution of gas on addition of acid; nitrate content greatly diminished;
Strong formation of nitrite.

158. Bacillus ruber of Kiel: good growth; vigorous evolution of gas on addition of acid; nitrate content greatly diminished;
Strong formation of nitrite.

159. Spirillum Rugala: moderate growth; no evolution of gas on addition of acid; nitrate content unchanged;
No formation of nitrite.

160. Bacterium rubilum: fair growth; no evolution of gas on addition of acid; nitrate content unchanged;
No formation of nitrite.

161. Bacillus ruminatus: good growth; no evolution of gas on addition of acid; nitrate content unchanged;
No formation of nitrite.

162. Bacillus rutilus: good growth; moderate evolution of gas on addition of acid; nitrate content slightly diminished;
Weak formation of nitrite.

163. Bacillus rutilensis: fair growth; moderate evolution of gas on addition of acid; nitrate content slighlty diminished;
Weak formation of nitrite.

164. Spirillum serpens: moderate growth; no evolution of gas on the addition of acid; nitrate content unchanged;
No formation of nitrite.

165. Bacillus silvaticus: good growth; vigorous evolution of gas on addition of acid; nitrate content greatly diminished;
Strong formation of nitrite.

166. Bacillus simplex: good growth; vigorous evolution of gas on addition of acid; nitrate content greatly diminished;
Strong formation of nitrite.

167. Vibrio saprophilus: good growth; vigorous evolution of gas on addition of acid; nitrate content greatly diminished;
Strong formation of nitrite.

168. Micrococcus sordidus: moderate growth; slight evolution of gas on addition of acid; nitrate content diminished;
Weak formation of nitrite.

169. Bacillus luteus sporogenes: good growth; no evolution of gas on addition of acid; nitrate content unchanged;
No formation of nitrite.

170. Bacterium der sorbose: good growth; vigorous evolution of gas on addition of acid; nitrate content greatly diminished;
Strong formation of nitrite.

171. Bacillus solanisparus: good growth; vigorous evolution of gas on addition of acid; nitrate content greatly diminished;
Strong formation of nitrite.

172. Bacillus sphaericus: good growth; no evolution of gas on addition of acid; nitrate content unchanged;
No formation of nitrite.

173. Staphlococcus cereus aureus: good growth; moderate evolution of gas on addition of acid; nitrate content slightly diminished;
Weak formation of nitrite.

174. Staphlococcus pyogenes citreus: good growth; no evolution of gas on addition of acid; nitrate content unchanged;
No formation of nitrite.

175. Staphlococcus pyogenes albus: good growth; moderate evolution of gas on addition of acid; nitrate content slightly reduced;
Weak formation of nitrite.

176. Staphlococcus pyogenes aureus: good growth; fair evolution of gas on addition of acid; nitrate content slightly diminished;
Weak formation of nitrite.

177. Bacillus ochraceus subflavus: good growth; vigorous evolution of gas on addition of acid; nitrate content greatly diminished;
Strong furmation of nitrite.

178. Bacterium subflavum: fair growth; no evolution of gas on addition of acid; nitrate content unchanged;
No formation of nitrite.

179. Micrococcus sulfur: moderate growth; no evolution of gas on addition of acid; nitrate content unchanged;
No formation of nitrite.

180. Bacillus subtilis: good growth; moderate evolution of gas on addition of acid; nitrate content slightly diminished;
Weak furmation of nitrite.

181. Bacterium Stutzeri: good growth; strong evolution of gas on addition of acid; nitrate content greatly diminished;
Strong formation of nitrite.

182. Bacillus synxanthus: good growth; moderate evolution of gas on addition of acid; nitrate content moderately diminished;
Weak formation of nitrite.

183. Bacterium tremellioides: good growth; vigorous evolution of gas on addition of acid; nitrate content greatly diminished;
Strong formation of nitrite.

184. Bacillus tumefaciens: fair growth; no evolution of gas on addition of acid; nitrate content unchanged;
No formation of nitrite.

185. Bacillus tumescens: good growth; no evolution of gas on addition of acid; nitrate content unchanged;
No formation of nitrite.

186. Bacillus typhosus: good growth; vigorous evolution of gas on addition of acid; nitrate content greatly diminished;
Strong formation of nitrite.

187. Bacillus para-typhosus: moderate growth; slight evolution of gas on addition of acid; nitrate content not greatly diminished;
Weak formation of nitrite.

188. Sarcina ventriculi: fair growth; moderate evolution of gas on addition of acid; nitrate content considerably diminished;
Moderate formation of nitrite.

189. Bacillus violaceus: moderate growth; fair evolution of gas on addition of acid; nitrate content slightly diminished;
Weak formation of nitrite.

190. Azotobacter vinelandi: good growth; moderate evolution of gas on addition of acid; nitrate content considerably diminished;
Weak formation of nitrite.

191. Micrococcus viticulosus: moderate growth; slight evolution of gas on addition of acid; nitrate content not greatly diminished;
Weak formation of nitrite.

192. Bacillus viridis: good growth; vigorous evolution of gas on addition of acid; nitrate content greatly diminished;
Strong formation of nitrite.

193. Bacillus aquatile villos: moderate growth; slight evolution of gas on addition of acid; nitrate content slightly diminished;
Weak formation of nitrite.

194. Bacillus vivax: fair growth; no evolution of gas on addition of acid; nitrate content unchanged;
No formation of nitrite.

195. Spirillum volutans: good growth; moderate evolution of gas on addition of acid; nitrate content slightly diminished;
Weak formation of nitrite.

196. Bacillus proteus vulgaris: good growth; vigorous evolution of gas on addition of acid; nitrate content greatly diminished;
Strong formation of nitrite.

197. Bacterium xanthochlorum: good growth; moderate evolution of gas on addition of acid; nitrate content slightly diminished;
Weak formation of nitrite.

198. Bacillus xylinum: good growth; moderate evolution of gas on addition of acid; nitrate content slightly diminished;
Weak formation of nitrite.

199. Bacillus proteus Zenkeri: good growth; moderate evolution of gas on addition of acid: nitrate content slightly diminished;
Weak formation of nitrite.

200. Bacillus Zopfii: scant growth; no evolution of gas on addition of acid; nitrate content unchanged;
No formation of nitrite.

201. Boden I (*Tsiklinsky-Sudpolar expedition, 1903-5*): good growth; vigorous evolution of gas on addition of acid; nitrate content greatly diminished;
Strong formation of nitrite.

## THE FOLLOWING ORGANISMS REDUCED NITRATE TO NITRITE

1. Bacterium acetosum; 2. Bacterium lactis aerogenes; 3. Bacillus brassicae acidae; 5. Bacillus acidi lactici; 6. Micrococcus albidus; 7. Bacillus amylovorus; 8. Bacillus anthracis; 10. Bacillus anthracoides; 11. Bacterium annulatum A; 12. Bacterium annulatum B; 13. Bacillus aquatile; 14. Bacillus arborescens; 15. Bacillus argentinensis; 16. Micrococcus ascoformans; 17. Bacillus asterosporus; 20. Bacillus baccarinii; 21. Bacterium beticolum; 22. Micrococcus brunneus; 23. Bacillus budapestiensis; 27. Monila candida; 30. Bacillus cereus; 31. Bacillus cereulens;. 34. Micrococcus cinnabareus; 35. Bacillus cloacae; 37. Bacillus constrictus; 39. Bacillus coli commune; 40. Bacillus coli-anaerogenes; 41. Bacillus carotovorus; 42. Bacillus cyanogenes; 43. Bacillus cylindrosporus; 44. Bacillus creusus; 47. Bacillus denitrificans; 49. Pseudomonas destructans; 50. Bacillus disciformans; 51. Bacillus enteritidis; 55. Bacterium lactis erythrogenes; 56. Bacillus ethacinicus; 57. Bacillus ethaceto succinus; 58. Bacillus ferruginous; 59. Bacillus faecalis alcaligenes; 60. Sarcina flava; 61. Micrococcus flavus; 62. Bacillus flavidus; 63. Bacterium filiforme; 64. Bacterium filifaciens; 65. Bacillus Fitzianus; 66. Bacillus fluorescens liquefaciens; 67. Bacillus fluorescens non liquefaciens; 69. Bacillus Frostii; 73. Bacillus aquatile griseum; 74. Micrococcus grossus; 75. Bacterium Hartlebi; 76. Bacillus Havaniensis; 77. Bacillus herbicoli aureus; 78. Bacillus helvolus; 79. Bacillus Hoagii; 81. Bacillus immobile; 82. Bacillus indicus; 83. Bacillus indigoferus; 84. Bacillus irritans; 85. Bacillus ivilans; 86. Bacillus jasminocyaneus; 89. Bacillus lactis; 90. Bacillus lactorubefaciens; 91. Bacillus lateritia; 93. Bacillus lactis amari liquefaciens; 94. Bacillus liodermos; 95. Bacillus limosus; 98. Bacillus lactis niger; 99. Bacillus liquefaciens niger; 100. Bacillus loxosus; 101. Bacterium aquatile gasformans non liquefaciens; 106. Bacillus melonis; 107. Bacillus mesentericus fuscus; 108. Bacillus mesentericus niger; 109. Bacillus mesentericus ruber; 110. Bacillus mesentericus vulgatus; 112. Bacillus miniaceus; 114. Sarcina mobilis, 115. Moeller's grass bacillus, Mist.; 117. Bacillus mycoides;

118. Bacillus nanus; 120. Bacillus oleraceae; 121. Bacillus olfactorius; 123. Bacillus oleae; 126. Bacillus oxalatus; 127. Bacterium paracoli gasformans anindolicum; 128. Bacillus parvus; 129. Rhodobacillus palustis; 131. Bacterium Petroselini; 132. Bacillus prodigiosus; 133. Bacillus lactis proteolyticus; 134. Bacillus plicatus; 136. Bacillus proteus; 138. Bacillus punctatus; 140. Bacillus phosphorescens; 141. Pseudomanas pyocyanea; 143. Pseudomonas radicicola clover; 146. Micrococcus roseus; 147. Bacillus of ropy milk; 148. Micrococcus rhodochrous; 149. Bacillus brunneus mycoides roseus; 150. Bacillus capsulatus roseus; 151. Bacillus ruber; 152. Micrococcus ruber; 153. Bacillus subtilis var ruber; 154. Bacillus ruber Plymouth; 157. Bacterium rugosum; 158. Bacillus ruber of Kiel; 162. Bacillus rutilus; 163. Bacillus rutilensis; 165. Bacillus silvaticus; 166. Bacillus simplex; 167. Vibrio saprophilus; 168. Micrococcus sordidus; 170. Bacterium der sorbose; 171. Bacillus solanisparus; 173. Staphlococcus cereus aureus; 175. Staphlococcus pyogenes albus; 176. Staphlococcus pyogenes aureus; 177. Bacillus ochraceus subflavus; 180. Bacillus subtilis; 181. Bacterium Stutzeri; 182. Bacillus synxanthus; 183. Bacterium tremellioides; 186. Bacillus typhosus; 187. Bacillus paratyphosus; 188. Sarcina ventriculi; 189. Bacillus violaceus; 190. Azotobacter vinelandii; 191. Micrococcus viticulosus; 192. Bacillus proteus viridis; 193. Bacillus aquatile villos; 195. Spirillum volutans; 196. Bacillus proteus vulgaris; 197. Bacterium xanthochlorum; 198. Bacillus xylinum; 199. Bacillus proteus Zenkeri; 201. Boden I. (Tsiklinsky Südpolarexpedition 1903—5).

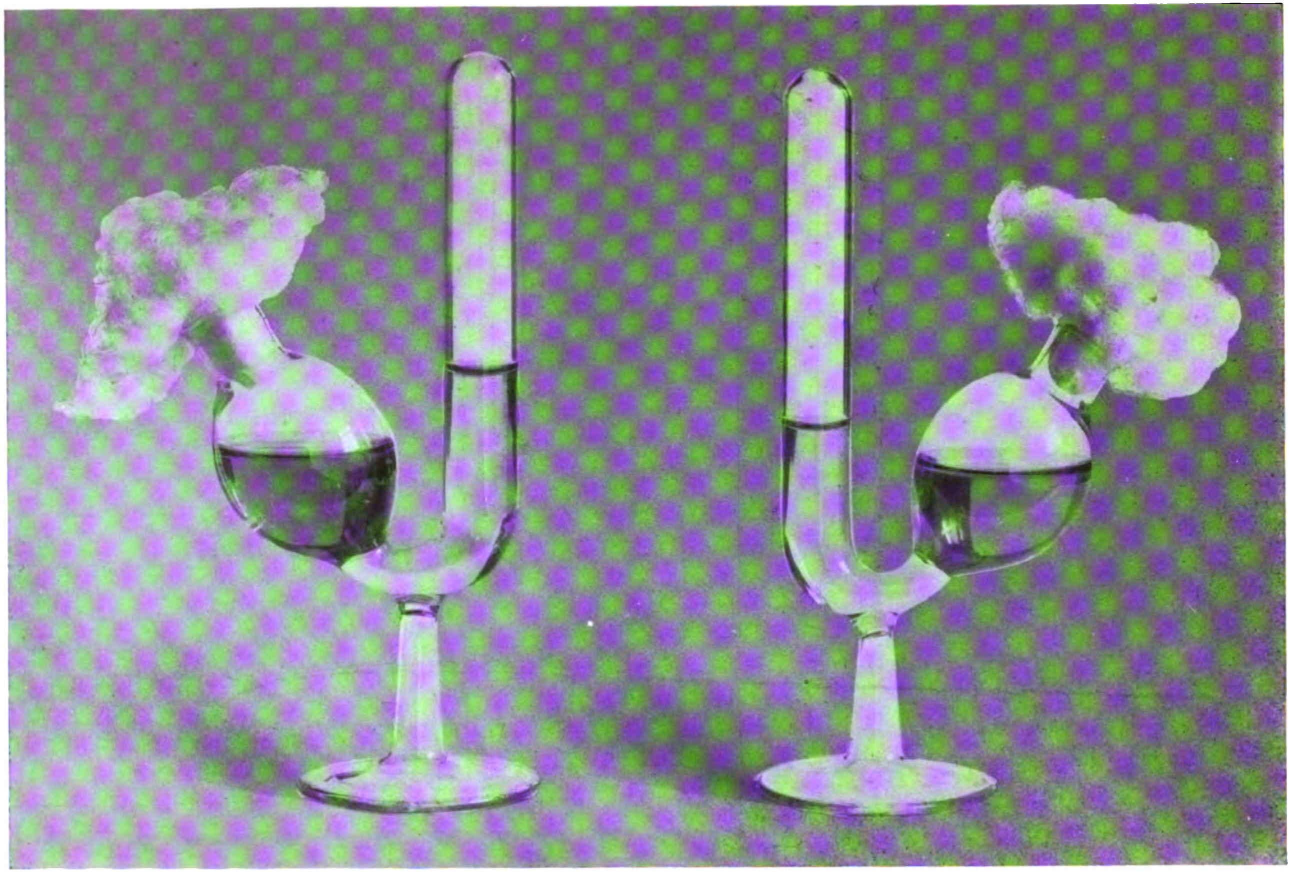
## THE FOLLOWING ORGANISMS DID NOT REDUCE NITRATE TO NITRITE

4. Micrococcus agilis; 9. Bacillus pseudo-anthracis; 18. Bacterium aurantiacus; 19. Sarcina aurantiaca; 24. Bacillus butyricus; 25. Bacillus candicans; 26. Micrococcus candicans; 28. Bacillus campestris; 29. Rhodobacillus capsulatus; 32. Micrococcus cereus; 33. Micrococcus carneus; 36. Micrococcus citreus; 38. Micrococcus concentricus; 45. Bacillus cyaneus; 46. Bacterium crysogloia; 48. Bacillus dendroides;

51. Bacillus esterigenes; 53. Bacillus esterigenes A; 54. Bacillus esterigenes D; 68. Bacillus fluorescens tenuis; 70. Bacillus fuchsinus; 71. Sarcina gasformans; 72. Bacillus graviolens; 80. Bacillus hyponitrous; 87. Bacillus juglandis; 88. Bacillus kiliensis; 92. Bacillus levans; 96. Sarcina liquefaciens; 97. Bacillus liquefaciens; 102. Micrococcus luteus; 103. Sarcina lutea; 104. Streptococcus luteus liquefaciens; 105. Bacillus maidis; 111. Bacillus megatherium; 113. Bacillus proteus mirabilis; 116. Bacterium muris; 119. Bacillus ochraceus; 122. Oidium lactis; 124. Cladothrix odorifera; 125. Cladothrix dichotoma; 130. Bacillus Petasites; 135. Bacterium phytophtorum; 137. Bacillus pumilis; 139. Bacillus fluorescens putidus; 142. Bacterium radiatum; 144. Bacillus ramosus non liquefaciens; 145. Bacillus rosaceus; 155. Bacillus rubidus; 156. Spirillum rubrum; 159. Spirillum Rugula; 160. Bacterium rubilum; 161. Bacillus ruminatus; 164. Spirillum serpens; 169. Bacterium luteus sporogenes; 172. Bacillus sphaericus; 174. Staphlococcus pyogenes citreus; 178. Bacterium subflavum; 179. Micrococcus sulfur; 184. Bacillus tumefaciens; 185. Bacillus tumescens; 194. Bacillus vivax; 200. Bacillus Zopfii.

Of the 201 organisms under consideration 139, or 69.1%, reduced nitrate to nitrite, and 62, or 30.9%, did not effect this reduction. Those organisms which produce green pigment almost invariably reduce nitrate to free nitrogen. This reduction takes place very rapidly, after forty-eight hours no nitrite remains in solutions of small concentration. It is impossible to declare from the vigor of the growth of the organism respecting its ability to effect the reduction of nitrate to nitrite. However, those bacteria which failed to perform such reduction were commonly found among those whose growth was slow and at best feeble.

Many organisms were inoculated into Giltay's medium in the hope that it would prove available for reduction experiments. This proved to be the case with soil inoculation, but in pure culture the slow growth of all and the refusal of many bacteria to develop in this synthetic medium, did not prove encouraging. Calcium glycerophosphate and calcium lactophosphate were also tried in this connection. These compounds proved to be unstable in solution and were difficult



Denitrification: Soil infusion inoculation.

to sterilize intact. Nothing was found superior to peptone although this medium in the absence of mineral salts and carbonaceous material is far from the optimum requirement of most bacteria.

## DENITRIFICATION

It must be evident that of all the organisms which have been studied, comparatively few reduce nitrite to free nitrogen in pure culture. Of the seventy soils under consideration, all of those which would be considered as suitable for crop production reduced nitrite to free nitrogen in a very short period of time. It must therefore be concluded that either the very few species of bacteria which effect such reduction are universally distributed, or that those organisms which will not perform this function in pure culture will work in symbiosis to effect this end. The latter phenomenon has been established with reference to a few organisms and will doubtless be extended to include a great variety. The operation of this function is not confined to nitrogenous media but is performed with equal vigor in Ashby's medium in which either glucose or mannite are employed. The reduction seemingly taking place a little slower in the case of glucose. In simple soil infusion of considerable concentration I have been unable to detect the slightest evidence of reduction of nitrates or nitrites on addition of these salts. That these reductions are effected wholly according to the nascent hydrogen theory seems improbable. By soil inoculation of the media I have been unable to reduce sulphates to hydrogen sulphide except in a very few instances, while with sewage sludge no difficulty is experienced. The phosphates also seem refractory. A selective action involving energy, nutrition, etc., may be concerned.

But eight species of bacteria are commonly cited as reducing nitrites to free nitrogen in pure culture: Bacterium centropunctatum, (H. Jensen), Bacterium filifaciens (H. Jensen), Bacterium Hartlebi (H. Jensen), Bacterium nitrovorum (H. Jensen), Pseudomonas pyocyanea (Migula), Bacillus denitrificans (H. Jensen) and probably two members of the Fluorescens group: Bacillus fluorescens and Bacillus fluorescens liquefaciens. Maassen observed that Bacillus praepollens broke down nitrates to free nitrogen only in sym-

biosis with other bacteria, and that nitrites were not reduced to free nitrogen except in harmony with organisms which reduced nitrates to nitrites. He discovered that the following organisms, in coöperation with Bacillus praepollens, would effect this reduction: "B. acidi lactici, B. capsulatus, B. cremoides, B. cuniculicida mobilis, B. diphtheria columbarum, B. enteritidis Gartneri, B. from lean meat, B. indigonaceus, B. mycoides, B. mesentericus ruber, B. mesentericus Flugge I, III and VII, B. mustelae septicus, B. miniaceus, B. prodigiosus, B. pneumoniae, B. proteus mirabilis, B. proteus vulgaris, B. psitticosis, B. rhinoscleromatis, B. ruber of Kiel, B. ruber plymouth, B. ruber purpureus, B. suipestifer, B. Hog-cholera (Salmon), B. swine plague, B. typhi-abdominalis, B. typhi-murium, B. violaceus, B. coli commune I, II, III, IV, B. lactis aerogenes, B. phosphorescens, M. candicans, Staphlococcus pyogenes albus, Staphlococcus pyogenes aureus, Sarcina flava II, Vibrio Blankenese, Vibrio Mottlau II, Vibrio tyrogenes Deneke."

An attempt was made to isolate the organisms from the soil, which perform the function of reducing nitrates to free nitrogen. As far as possible all the organisms were isolated from soil number 9. None of these bacteria reduced nitrates to free nitrogen in pure culture. Of the fifteen different species thus isolated various combinations were made in the hope of discovering a symbiotic relation, but of the many combinations thus effected in no instance did I succeed in securing the desired result. After fishing the different colonies from a great number of plates, the agar in several was carefully rolled together with a sterile spatula and introduced into a medium prepared for the reduction of nitrates, those plates which contained the greater number of colonies invariably reduced the nitrate to free nitrogen, but in some of the plates on which were few colonies no reduction took place.

I am under obligations to Dr. A. F. MacLeod, Assistant Professor of Physical Chemistry in Beloit College, Beloit, Wisconsin, for valuable suggestions and assistance; and likewise to Dr. H. H. Waite, Professor of Bacteriology and Pathology in the University of Nebraska, for valuable suggestions and assistance.